

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/32, C07K 14/325, A01N 63/00, C12N 15/82		A2	(11) International Publication Number: WO 97/40162 (43) International Publication Date: 30 October 1997 (30.10.97)
(21) International Application Number: PCT/US97/06463 (22) International Filing Date: 18 April 1997 (18.04.97) (30) Priority Data: 08/633,993 19 April 1996 (19.04.96) US (71) Applicant: MYCOGEN CORPORATION [US/US]; 5501 Oberlin Drive, San Diego, CA 92121 (US). (72) Inventors: NARVA, Kenneth, E.; 12123 Caminito Mira Del Mar, San Diego, CA 92130 (US). SCHNEPP, H., Ernest; 7954 Handel Court, San Diego, CA 92126 (US). KNUTH, Mark; 16744 Valle Verde Road, Poway, CA 92074 (US). POLLARD, Michael, R.; 12358 Pathos Lane, San Diego, CA 92129 (US). CARDINEAU, Guy; 17041 Clouderost Drive, Poway, CA 92064 (US). SCHWAB, George, E.; 1351 Walnutview, Encinitas, CA 92024 (US). (74) Agents: SANDERS, Jay, M. et al.; Saliwanchik, Lloyd & Saliwanchik, P.A., Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606-6669 (US).			(81) Designated States: AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: PESTICIDAL TOXINS			
(57) Abstract <p>The subject invention concerns new classes of pesticidal toxins and polynucleotide sequences which encode these toxins. Also described are novel pesticidal isolates of <i>Bacillus thuringiensis</i>.</p>			

DESCRIPTION

PESTICIDAL TOXINS

Cross-Reference to a Related Application

This application is a continuation-in-part of Application Serial No. 08/633,993, filed April 19, 1996.

Background of the Invention

The soil microbe *Bacillus thuringiensis* (*B.t.*) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain *B.t.* toxin genes have been isolated and sequenced, and recombinant DNA-based *B.t.* products have been produced and approved for use. In addition, with the use of genetic engineering techniques, new approaches for delivering these *B.t.* endotoxins to agricultural environments are under development, including the use of plants genetically engineered with endotoxin genes for insect resistance and the use of stabilized intact microbial cells as *B.t.* endotoxin delivery vehicles (Gaertner, F.H., L. Kim [1988] *TIBTECH* 6:S4-S7). Thus, isolated *B.t.* endotoxin genes are becoming commercially valuable.

Until the last ten years, commercial use of *B.t.* pesticides has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of *B. thuringiensis* subsp. *kurstaki* have been used for many years as commercial insecticides for lepidopteran pests. For example, *B. thuringiensis* var. *kurstaki* HD-1 produces a crystalline δ -endotoxin which is toxic to the larvae of a number of lepidopteran insects.

In recent years, however, investigators have discovered *B.t.* pesticides with specificities for a much broader range of pests. For example, other species of *B.t.*, namely *israelensis* and *tenebrionis* (a.k.a. *B.t.* M-7, a.k.a. *B.t. san diego*), have been used commercially to control insects of the orders Diptera and Coleoptera, respectively (Gaertner, F.H. [1989] "Cellular Delivery Systems for Insecticidal Proteins: Living and Non-Living Microorganisms," in *Controlled Delivery of Crop Protection Agents*, R.M. Wilkins, ed., Taylor and Francis, New York and London, 1990, pp. 245-255). See also Couch, T.L. (1980) "Mosquito Pathogenicity of *Bacillus thuringiensis* var. *israelensis*," *Developments in Industrial Microbiology* 22:61-76; Beegle, C.C., (1978) "Use of Entomogenous Bacteria in Agroecosystems," *Developments in Industrial Microbiology* 20:97-104. Krieg, A., A.M. Huger, G.A. Langenbruch, W. Schnetter

and, ultimately, reductions in yield. Severe infestations can ruin an entire cutting of hay. The adults, also foliar feeders, cause additional, but less significant, damage.

Approximately 9.3 million acres of U.S. corn are infested with corn rootworm species complex each year. The corn rootworm species complex includes the northern corn rootworm, *Diabrotica barberi*, the southern corn rootworm, *D. undecimpunctata howardi*, and the western corn rootworm, *D. virgifera virgifera*. The soil-dwelling larvae of these *Diabrotica* species feed on the root of the corn plant, causing lodging. Lodging eventually reduces corn yield and often results in death of the plant. By feeding on cornsilks, the adult beetles reduce pollination and, therefore, detrimentally effect the yield of corn per plant. In addition, adults and larvae of the genus *Diabrotica* attack cucurbit crops (cucumbers, melons, squash, etc.) and many vegetable and field crops in commercial production as well as those being grown in home gardens.

Control of corn rootworm has been partially addressed by cultivation methods, such as crop rotation and the application of high nitrogen levels to stimulate the growth of an adventitious root system. However, chemical insecticides are relied upon most heavily to guarantee the desired level of control. Insecticides are either banded onto or incorporated into the soil. The major problem associated with the use of chemical insecticides is the development of resistance among the treated insect populations.

Brief Summary of the Invention

The subject invention concerns novel materials and methods for controlling non-mammalian pests. In a preferred embodiment, the subject invention provides materials and methods for the control of coleopteran pests. In specific embodiments, the materials and methods described herein are used to control alfalfa weevil and/or corn rootworm.

The subject invention advantageously provides two new classes of polynucleotide sequences which encode corresponding novel classes of pesticidal proteins. One novel class of polynucleotide sequences as described herein encodes toxins which have a full-length molecular weight of approximately 40-50 kDa. In a specific embodiment, these toxins have a molecular weight of about 43-47 kDa. A second class of polynucleotides, which encodes pesticidal proteins of about 10-15 kDa, is also provided according to the subject invention. In a specific embodiment, these toxins have a molecular weight of about 13-14 kDa. The subject invention concerns polynucleotides which encode the 40-50 kDa and 10-15 kDa toxins, polynucleotides which encode pesticidal fragments of the full length toxins, and polynucleotide sequences which encode longer forms of these toxins which include, for example, a protoxin region. In a

preferred embodiment, these toxins, including the fragments, are active against coleopteran pests.

Specific *B.t.* toxins useful according to the invention include toxins which can be obtained from the *B.t.* isolates designated as PS80JJ1, PS149B1, and PS167H2. Of these, PS149B1 and PS167H2 are novel isolates. The subject invention also includes the use of variants of the exemplified *B.t.* isolates and toxins which have substantially the same coleopteran-active properties as the specifically exemplified *B.t.* isolates and toxins. Such variant isolates would include, for example, mutants. Procedures for making mutants are well known in the microbiological art. Ultraviolet light and chemical mutagens such as nitrosoguanidine are used extensively toward this end.

In one embodiment of the subject invention, the polynucleotide sequences of the subject invention are used to encode toxins of approximately 43-47 kDa. These toxins are then used to control coleopteran pests. In a particularly preferred embodiment, the coleopteran pests are corn rootworms. The genes which encode the 43-47 kDa toxins can be obtained from, for example, PS80JJ1, PS149B1, or PS167H2. In a second embodiment, toxins of approximately 13-14 kDa are used to control coleopteran pests. The approximately 13-14 kDa toxin, as well as the genes which encode these toxins, can also be obtained from PS80JJ1, PS149B1, or PS167H2. In a particularly preferred embodiment, the activity of the 43-47 kDa toxins can be augmented and/or facilitated by further contacting the target pests with an approximately 13-14 kDa toxin.

In a preferred embodiment, the subject invention concerns plants cells transformed with at least one polynucleotide sequence of the subject invention such that the transformed plant cells express pesticidal toxins in tissues consumed by the target pests.

Alternatively, the *B.t.* isolates of the subject invention, or recombinant microbes expressing the toxins described herein, can be used to control pests. In this regard, the invention includes the treatment of substantially intact *B.t.* cells, and/or recombinant cells containing the expressed toxins of the invention, treated to prolong the pesticidal activity when the substantially intact cells are applied to the environment of a target pest. The treated cell acts as a protective coating for the pesticidal toxin. The toxin becomes active upon ingestion by a target insect.

Brief Description of the Drawings

Figure 1 shows three specific 43-47 kDa pesticidal toxins of the subject invention as well as a consensus sequence for these pesticidal toxins.

Figure 2 shows the relationship of the 14 and 45 kDa sequences of PS80JJ1 (SEQ ID NOS. 31 and 10).

SEQ ID NO. 17 is a peptide sequence used in primer design according to the subject invention.

SEQ ID NO. 18 is a peptide sequence used in primer design according to the subject invention.

5 SEQ ID NO. 19 is a peptide sequence used in primer design according to the subject invention.

16. SEQ ID NO. 20 is a nucleotide sequence corresponding to the peptide of SEQ ID NO.

10 17. SEQ ID NO. 21 is a nucleotide sequence corresponding to the peptide of SEQ ID NO.

18. SEQ ID NO. 22 is a nucleotide sequence corresponding to the peptide of SEQ ID NO.

19. SEQ ID NO. 23 is a nucleotide sequence corresponding to the peptide of SEQ ID NO.

15 22. SEQ ID NO. 24 is a reverse primer based on the reverse complement of SEQ ID NO.

23. SEQ ID NO. 25 is a reverse primer based on the reverse complement of SEQ ID NO.

20 26. SEQ ID NO. 26 is a forward primer based on the PS80JJ1 44.3 kDa toxin.

SEQ ID NO. 27 is a reverse primer based on the PS80JJ1 44.3 kDa toxin.

SEQ ID NO. 28 is a generic sequence representing a new class of toxins according to the subject invention.

SEQ ID NO. 29 is an oligonucleotide probe used according to the subject invention.

25 SEQ ID NO. 30 is the nucleotide sequence of the entire genetic locus containing open reading frames of both the 14 and 45 kDa PS80JJ1 toxins and the flanking nucleotide sequences.

SEQ ID NO. 31 is the nucleotide sequence of the PS80JJ1 14 kDa toxin open reading frame.

SEQ ID NO. 32 is the deduced amino acid sequence of the 14 kDa toxin of PS80JJ1.

30 SEQ ID NO. 33 is a reverse oligonucleotide primer used according to the subject invention.

SEQ ID NO. 34 is the nucleotide sequence of the entire genetic locus containing open reading frames of both the 14 and 44 kDa PS167H2 toxins and the flanking nucleotide sequences.

weight. Therefore, reference herein to, for example, a 45 kDa protein or a 14 kDa protein is understood to refer to proteins of approximately that size even if the true molecular weight is somewhat different.

The subject invention concerns not only the polynucleotide sequences which encode these classes of toxins, but also the use of these polynucleotide sequences to produce recombinant hosts which express the toxins. In a further aspect, the subject invention concerns the combined use of an approximately 40-50 kDa toxin of the subject invention together with an approximately 10-15 kDa toxin of the subject invention to achieve highly effective control of pests, including coleopterans such as corn rootworm.

A further aspect of the subject invention concerns two novel isolates and the toxins and genes obtainable from these isolates. The novel *B.t.* isolates of the subject invention have been designated PS149B1 and PS167H2.

The new classes of toxins and polynucleotide sequences provided here are defined according to several parameters. One critical characteristic of the toxins described herein is pesticidal activity. In a specific embodiment, these toxins have activity against coleopteran pests. The toxins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules within each novel class can be defined herein in terms of homology to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain exemplified probes and primers. The classes of toxins provided herein can also be identified based on their immunoreactivity with certain antibodies and based upon their adherence to a generic formula.

The sequence of three approximately 45 kDa toxins of the subject invention are provided as SEQ ID NOS. 11, 43, and 38. In a preferred embodiment of the subject invention, the toxins in this new class have a sequence which conforms to the generic sequence presented as SEQ ID NO. 28. In a specific embodiment, the toxins of this class will conform to the consensus sequence shown in Figure 1.

In a preferred embodiment, the toxins of the subject invention have at least one of the following characteristics:

- (a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes

characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

- (h) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33; and
- (i) said toxin comprises an amino acid sequence which has at least about 60% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of SEQ ID NO. 41.

As used herein "stringent" conditions for hybridization refers to conditions which achieve the same, or about the same, degree of specificity of hybridization as the conditions employed by the current applicants. Specifically, hybridization of immobilized DNA on Southern blots with ³²P-labeled gene-specific probes was performed by standard methods (Maniatis, T., E.F. Fritsch, J. Sambrook [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.). In general, hybridization and subsequent washes were carried out under stringent conditions that allowed for detection of target sequences with homology to the PS80JJ1 toxin genes. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25° C below the melting temperature (T_m) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz, G.A., K.A. Jacobs, T.H. Eickbush, P.T. Cherbas, and F.C. Kafatos [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

$$T_m = 81.5^\circ \text{C} + 16.6 \log[\text{Na}^+] + 0.41(\% \text{G} + \text{C}) - 0.61(\% \text{formamide}) - 600/\text{length of duplex}$$
in base pairs.

Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).
- (2) Once at T_m-20°C for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

For oligonucleotide probes, hybridization was carried out overnight at 10-20°C below the melting temperature (T_m) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. T_m for oligonucleotide probes was determined by the following formula:

should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of a deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures. The depositor acknowledges the duty to replace the deposit(s) should the depository be unable to furnish a sample when requested, due to the condition of the deposit(s). All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

Following is a table which provides characteristics of certain *B.t.* isolates useful according to the subject invention.

Table 1. Description of *B.t.* strains toxic to coleopterans

Culture	Crystal Description	Approx. MW (kDa)	Serotype	NRRL Deposit	Deposit Date
PS80JJ1	multiple attached	130, 90, 47, 37, 14	4a4b, sotto	B-18679	7-17-90
PS149B1		130, 47, 14		B-21553	3-28-96
PS167H2		70, 47, 14		B-23554	3-28-96

Genes and toxins. The genes and toxins useful according to the subject invention include not only the full length sequences disclosed but also fragments of these sequences, variants, mutants, and fusion proteins which retain the characteristic pesticidal activity of the toxins specifically exemplified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences which encode the same toxins or which encode equivalent toxins having pesticidal activity. As used herein, the term "equivalent toxins" refers to toxins having the same or essentially the same biological activity against the target pests as the claimed toxins.

fluorescent as described in International Application No. WO93/16094. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed that the probe and sample have substantial homology. Preferably, hybridization is conducted under stringent conditions by techniques well-known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170. Detection of the probe provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying toxin-encoding genes of the subject invention. The nucleotide segments which are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures. These nucleotide sequences can also be used as PCR primers to amplify genes of the subject invention.

Certain toxins of the subject invention have been specifically exemplified herein. Since these toxins are merely exemplary of the toxins of the subject invention, it should be readily apparent that the subject invention comprises variant or equivalent toxins (and nucleotide sequences coding for equivalent toxins) having the same or similar pesticidal activity of the exemplified toxin. Equivalent toxins will have amino acid homology with an exemplified toxin. The amino acid identity will typically be greater than 60%, preferably be greater than 75%, more preferably greater than 80%, more preferably greater than 90%, and can be greater than 95%. The amino acid homology will be highest in critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 2 provides a listing of examples of amino acids belonging to each class.

Xanthomonas, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, e.g., genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacterium tumefaciens*, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium melioli*, *Alcaligenes entrophus*, and *Azotobacter vinlandii*; and phytosphere yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces roseus*, *S. odoratus*, *Kluyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

A wide variety of ways are available for introducing a *B.t.* gene encoding a toxin into a microorganism host under conditions which allow for stable maintenance and expression of the gene. These methods are well known to those skilled in the art and are described, for example, in United States Patent No. 5,135,867, which is incorporated herein by reference.

Control of coleopterans, including corn rootworm using the isolates, toxins, and genes of the subject invention can be accomplished by a variety of methods known to those skilled in the art. These methods include, for example, the application of *B.t.* isolates to the pests (or their location), the application of recombinant microbes to the pests (or their locations), and the transformation of plants with genes which encode the pesticidal toxins of the subject invention. Recombinant microbes may be, for example, a *B.t.*, *E. coli*, or *Pseudomonas*. Transformations can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

Synthetic genes which are functionally equivalent to the toxins of the subject invention can also be used to transform hosts. Methods for the production of synthetic genes can be found in, for example, U.S. Patent No. 5,380,831.

Control of other pests such as lepidopterans and other insects, nematodes, and mites can also be accomplished by those skilled in the art using standard techniques combined with the teachings provided herein.

Treatment of cells. As mentioned above, *B.t.* or recombinant cells expressing a *B.t.* toxin can be treated to prolong the toxin activity and stabilize the cell. The pesticide microcapsule that is formed comprises the *B.t.* toxin within a cellular structure that has been stabilized and will protect the toxin when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or eukaryotes, normally being

packaging or formation of inclusion bodies; survival in aqueous environments; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

5 Growth of cells. The cellular host containing the *B.t.* insecticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B.t.* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

10 The *B.t.* cells of the invention can be cultured using standard art media and fermentation techniques. Upon completion of the fermentation cycle the bacteria can be harvested by first separating the *B.t.* spores and crystals from the fermentation broth by means well known in the art. The recovered *B.t.* spores and crystals can be formulated into a wettable powder, liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers, and other components to facilitate handling and application for particular target pests.

15 These formulations and application procedures are all well known in the art.

Formulations. Formulated bait granules containing an attractant and spores and crystals of the *B.t.* isolates, or recombinant microbes comprising the genes obtainable from the *B.t.* isolates disclosed herein, can be applied to the soil. Formulated product can also be applied as

20 a seed-coating or root treatment or total plant treatment at later stages of the crop cycle. Plant and soil treatments of *B.t.* cells may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing

25 agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

 As would be appreciated by a person skilled in the art, the pesticidal concentration will

30 vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4

Example 1 - Culturing of *B.t.* Isolates of the Invention

A subculture of the *B.t.* isolates, or mutants thereof, can be used to inoculate the following medium, a peptone, glucose, salts medium.

5	Bacto Peptone	7.5 g/l
	Glucose	1.0 g/l
	KH ₂ PO ₄	3.4 g/l
	K ₂ HPO ₄	4.35 g/l
	Salt Solution	5.0 ml/l
10	CaCl ₂ Solution	5.0 ml/l
	pH 7.2	
	Salts Solution (100 ml)	
	MgSO ₄ ·7H ₂ O	2.46 g
15	MnSO ₄ ·H ₂ O	0.04 g
	ZnSO ₄ ·7H ₂ O	0.28 g
	FeSO ₄ ·7H ₂ O	0.40 g
	CaCl ₂ Solution (100 ml)	
20	CaCl ₂ ·2H ₂ O	3.66 g

The salts solution and CaCl₂ solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30°C on a rotary shaker at 200 rpm for 64 hr.

25 The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

The *B.t.* spores and/or crystals, obtained in the above fermentation, can be isolated by procedures well known in the art. A frequently-used procedure is to subject the harvested fermentation broth to separation techniques, e.g., centrifugation.

30

Example 2 - Protein Purification for 45 kDa 80JJ1 Protein

One gram of lyophilized powder of 80JJ1 was suspended in 40 ml of buffer containing 80 mM Tris-Cl pH 7.8, 5 mM EDTA, 100 µM PMSF, 0.5 µg/ml Leupeptin, 0.7 µg/ml Pepstatin, and 40 µg/ml Bestatin. The suspension was centrifuged, and the resulting supernatant was

Example 3 - Purification of the 14 kDa Peptide of PS80J11

0.8 ml of the white dialysis suspension (approximately 21 mg/ml) containing the 47 kDa, 45 kDa, and 15 kDa peptides, was dissolved in 10 ml of 40% NaBr, and 0.5 ml of 100 mM EDTA were added. After about 18 hours (overnight), a white opaque suspension was obtained. This was collected by centrifugation and discarded. The supernatant was concentrated in a Centricon-30 (Amicon Corporation) to a final volume of approximately 15 ml. The filtered volume was washed with water by filter dialysis and incubated on ice, eventually forming a milky white suspension. The suspension was centrifuged and the pellet and supernatant were separated and retained. The pellet was then suspended in 1.0 ml water (approximately 6 mg/ml). The pellet contained substantially pure 15 kDa protein when analyzed by SDS-PAGE.

The N-terminal amino acid sequence was determined to be: Ser-Ala-Arg-Glu-Val-His-Ile-Glu-Ile-Asn-Asn-Thr-Arg-His-Thr-Leu-Gln-Leu-Glu-Ala-Lys-Thr-Lys-Leu (SEQ ID NO. 3).

Example 4 - Protein Purification and Characterization of PS149B1 45 kDa Protein

The P1 pellet was resuspended with two volumes of deionized water per unit wet weight, and to this was added nine volumes of 40% (w/w) aqueous sodium bromide. This and all subsequent operations were carried out on ice or at 4-6°C. After 30 minutes, the suspension was diluted with 36 volumes of chilled water and centrifuged at 25,000 x g for 30 minutes to give a pellet and a supernatant.

The resulting pellet was resuspended in 1-2 volumes of water and layered on a 20-40% (w/w) sodium bromide gradient and centrifuged at 8,000 x g for 100 minutes. The layer banding at approximately 32% (w/w) sodium bromide (the "inclusions", or INC) was recovered and dialyzed overnight against water using a dialysis membrane with a 6-8 kDa MW cut-off. Particulate material was recovered by centrifugation at 25,000 x g, resuspended in water, and aliquoted and assayed for protein by the method of Lowry and by SDS-PAGE.

The resulting supernatant was concentrated 3- to 4-fold using Centricon-10 concentrators, then dialyzed overnight against water using a dialysis membrane with a 6-8 kDa MW cut-off. Particulate material was recovered by centrifugation at 25,000 x g, resuspended in water, and aliquoted and assayed for protein by the method of Lowry and by SDS-PAGE. This fraction was denoted as P1.P2.

The peptides in the pellet suspension were separated using SDS-PAGE (Laemmli, U.K., *supra*) in 15% acrylamide gels. The separated proteins were then electrophoretically blotted to a PVDF membrane (Millipore Corp.) in 10 mM CAPS pH 11.0, 10% MeOH at 100 V constant.

1) and to the sequence obtained for the 14 kDa peptide obtained from 80JJ1 spore/crystal powders with the N-terminal sequence (SEQ ID NO. 3).

Clearly, the 45-47 kDa proteins are highly related and probably represent one gene family, and the 14 kDa proteins are highly related and probably represent another gene family.

5
Example 6 - Molecular Cloning, Expression, and DNA Sequence Analysis of a Novel δ -Endotoxin Gene from *Bacillus thuringiensis* Strain PS80JJ1

10
Total cellular DNA was prepared from *Bacillus thuringiensis* (B.t.) cells grown to an optical density, at 600 nm, of 1.0. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/ml lysozyme in 0.3 M sucrose, 25 mM Tris-Cl [pH 8.0], 25 mM EDTA). After incubation at 37°C for 1 hour, protoplasts were lysed by two cycles of freezing and thawing. Nine volumes of a solution of 0.1 M NaCl, 0.1% SDS, 0.1 M Tris-Cl were added to complete lysis. The cleared lysate was extracted twice with phenol:chloroform (1:1). Nucleic acids were precipitated with two volumes of ethanol and pelleted by centrifugation. The pellet was resuspended in TE buffer and RNase was added to a final concentration of 50 µg/ml. After 15
incubation at 37°C for 1 hour, the solution was extracted once each with phenol:chloroform (1:1) and TE-saturated chloroform. DNA was precipitated from the aqueous phase by the addition of one-tenth volume of 3 M NaOAc and two volumes of ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer.

20
An oligonucleotide probe for the gene encoding the PS80JJ1 45 kDa toxin was designed from N-terminal peptide sequence data. The sequence of the 29-base oligonucleotide probe was: 5'-ATG YTW GAT ACW AAT AAA GTW TAT GAA AT-3' (SEQ ID NO. 8)
This oligonucleotide was mixed at four positions as shown. This probe was radiolabeled with ³²P and used in standard condition hybridization of Southern blots of PS80JJ1 total cellular DNA 25
digested with various restriction endonucleases. Representative autoradiographic data from these experiments showing the sizes of DNA restriction fragments containing sequence homology to the 44.3 kDa toxin oligonucleotide probe of SEQ ID NO. 8 are presented in Table 3.

An oligonucleotide probe for the gene encoding the PS80JJ1 14 kDa toxin was designed from N-terminal peptide sequence data. The sequence of the 28-base oligonucleotide probe was: 5' GW GAA GTW CAT ATW GAA ATW AAT AAT AC 3' (SEQ ID NO. 29). This oligonucleotide was mixed at four positions as shown. The probe was radiolabelled with ³²P and used in standard condition hybridizations of Southern blots of PS80JJ1 total cellular and pMYC2421 DNA digested with various restriction endonucleases. These RFLP mapping experiments demonstrated that the gene encoding the 14 kDa toxin is located on the same genomic *EcoRI* fragment that contains the N-terminal coding sequence for the 44.3 kDa toxin.

To test expression of the PS80JJ1 toxin genes in *B.t.*, pMYC2420 was transformed into the acrySTALLIFEROUS (Cry-) *B.t.* host, CryB (A. Aronson, Purdue University, West Lafayette, IN), by electroporation. Expression of both the approximately 14 and 44.3 kDa PS80JJ1 toxins encoded by pMYC2420 was demonstrated by SDS-PAGE analysis. Toxin crystal preparations from the recombinant CryB[pMYC2420] strain, MR536, were assayed and found to be active against western corn rootworm.

The PS80JJ1 toxin genes encoded by pMYC2421 were sequenced using the ABI373 automated sequencing system and associated software. The sequence of the entire genetic locus containing both open reading frames and flanking nucleotide sequences is shown in SEQ ID NO. 30. The termination codon of the 14 kDa toxin gene is 121 base pairs upstream (5') from the initiation codon of the 44.3 kDa toxin gene (Figure 2). The PS80JJ1 14 kDa toxin open reading frame nucleotide sequence (SEQ ID NO. 31), the 44.3 kDa toxin open reading frame nucleotide sequence (SEQ ID NO. 10), and the respective deduced amino acid sequences (SEQ ID NO. 32 and SEQ ID NO. 11) are novel compared to other toxin genes encoding pesticidal proteins.

Thus, the nucleotide sequence encoding the 14 kDa toxin of PS80JJ1 is shown in SEQ ID NO. 31. The deduced amino acid sequence of the 14 kDa toxin of PS80JJ1 is shown in SEQ ID NO. 32. The nucleotide sequences encoding both the 14 and 45 kDa toxins of PS80JJ1, as well as the flanking sequences, are shown in SEQ ID NO. 30. The relationship of these sequences is shown in Figure 2.

A subculture of *E. coli* NM522 containing plasmid pMYC2421 was deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, IL 61604 USA on March 28, 1996. The accession number is NRRL B-21555.

Each of the three strains exhibited unique RFLP patterns. The hybridizing DNA fragments from PS149B1 or PS167H2 contain all or part of toxin genes with sequence homology to the PS80JJ1 44.3 kDa toxin.

Table 5. Restriction fragment length polymorphisms of PS80JJ1, PS149B1, and PS167H2 cellular DNA fragments on Southern blots that hybridized with the PS80JJ1 14 kDa toxin oligonucleotide probe under standard conditions

Restriction enzyme	Strain		
	PS80JJ1	PS149B1	PS167H2
Approximate fragment size (kbp)			
<i>EcoRI</i>	5.6	2.7	2.7
<i>HindIII</i>	7.1	6.0	4.7
<i>XbaI</i>	8.4	11.2	11.2

Each of the three strains exhibited unique RFLP patterns. The hybridizing DNA fragments from PS149B1 or PS167H2 contain all or part of toxin genes with sequence homology to the PS80JJ1 14 kDa toxin gene.

Portions of the toxin genes in PS149B1 or PS167H2 were amplified by PCR using forward and reverse oligonucleotide primer pairs designed based on the PS80JJ1 44.3 kDa toxin gene sequence. For PS149B1, the following primer pair was used:

Forward:

5'-ATG YTW GAT ACW AAT AAA GTW TAT GAA AT-3' (SEQ ID NO. 8)

Reverse:

5'-GGA TTA TCT ATC TCT GAG TGT TCT TG-3' (SEQ ID NO. 9)

For PS167H2, the same primer pair was used. These PCR-derived fragments were sequenced using the ABI373 automated sequencing system and associated software. The partial gene and peptide sequences obtained are shown in SEQ ID NO. 12-15. These sequences contain portions of the nucleotide coding sequences and peptide sequences for novel corn rootworm-active toxins present in *B.t.* strains PS149B1 or PS167H2.

vector, pHT370 (Arantes, O., D. Lereclus [1991] *Gene* 108:115-119) for expression analyses in *Bacillus thuringiensis* (see below). The resultant recombinant, high copy number bifunctional plasmid was designated pMYC2429.

The PS149B1 toxin genes encoded by pMYC2429 were sequenced using the ABI automated sequencing system and associated software. The sequence of the entire genetic locus containing both open reading frames and flanking nucleotide sequences is shown in SEQ ID NO. 39. The termination codon of the 14 kDa toxin gene is 108 base pairs upstream (5') from the initiation codon of the 44 kDa toxin gene. The PS149B1 14 kDa toxin coding sequence (SEQ ID NO. 40), the 44 kDa toxin coding sequence (SEQ ID NO. 42), and the respective deduced amino acid sequences, SEQ ID NO. 41 and SEQ ID NO. 43, are novel compared to other known toxin genes encoding pesticidal proteins. The toxin genes are arranged in a similar manner as, and have some homology with, the PS80JJ1 and PS167H2 14 and 44 kDa toxins. Together, these three toxin operons comprise a new family of pesticidal toxins.

A subculture of *E. coli* NM522 containing plasmid pMYC2429 was deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 USA on 26 March 1997. The accession number is NRRL B-21673.

Example 9 – PCR Amplification for Identification and Cloning Novel Corn Rootworm-Active Toxin

The DNA and peptide sequences of the three novel approximately 45 kDa corn rootworm-active toxins from PS80JJ1, PS149B1, and PS167H2 (SEQ ID NOS. 12-15) were aligned with the Genetics Computer Group sequence analysis program Pileup using a gap weight of 3.00 and a gap length weight of 0.10. The sequence alignments were used to identify conserved peptide sequences to which oligonucleotide primers were designed that were likely to hybridize to genes encoding members of this novel toxin family. Such primers can be used in PCR to amplify diagnostic DNA fragments for these and related toxin genes. Numerous primer designs to various sequences are possible, four of which are described here to provide an example. These peptide sequences are:

Asp-Ile-Asp-Asp-Tyr-Asn-Leu (SEQ ID NO. 16)

Trp-Phe-Leu-Phe-Pro-Ile-Asp (SEQ ID NO. 17)

Gln-Ile-Lys-Thr-Thr-Pro-Tyr-Tyr (SEQ ID NO. 18)

Tyr-Glu-Trp-Gly-Thr-Glu (SEQ ID NO. 19).

The corresponding nucleotide sequences are:

When used in standard PCR reactions, this primer pair amplified a diagnostic 1390 bp DNA fragment that includes the entire 14 kDa toxin coding sequence and some 3' flanking sequences corresponding to the 121 base intergenic spacer and a portion of the 44.3 kDa toxin gene. When used in combination with the 14 kDa forward primer, PCR will generate a diagnostic 322 base pair DNA fragment.

Example 10 – Bioassay of Protein

A preparation of the insoluble fraction from the dialyzed NaBr extract of 80JJ1 containing the 47 kDa, 45 kDa, and 15 kDa peptides was bioassayed against Western corn rootworm and found to exhibit significant toxin activity.

Example 11 – Bioassay of Protein

The purified protein fractions from PS149B1 were bioassayed against western corn rootworm and found to exhibit significant toxin activity when combined. In fact, the combination restored activity to that noted in the original preparation (P1). The following bioassay data set presents percent mortality and demonstrates this effect.

Table 7.

Concentration ($\mu\text{g}/\text{cm}^2$)	P1	INC	P1.P2	INC + P1.P2
300	88, 100, 94	19	13	100
100	94, 50, 63	31	38	94
33.3	19, 19, 44	38	13	50
11.1	13, 56, 25	12	31	13
3.7	0, 50, 0	0	31	13
1.2	13, 43, 12	0	12	19
0.4	6, 12, 6	25	19	6

Example 12 – Clone Dose-Response Bioassays

The PS80JJ1 toxin operon was also subcloned from pMYC2421 into pHT370 for direct comparison of bioactivity with the recombinant toxins cloned from PS149B1 and PS167H2. The resultant recombinant, high copy number bifunctional plasmid was designated pMYC2426.

Genes encoding pesticidal toxins, as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for preparation for the insertion of foreign genes into higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the *B.t.* toxin can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted.

The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in EP 120 516; Hoekema (1985) In: *The Binary Plant Vector System*, Offset-durkkerij Kanters B.V., Alblaserdam, Chapter 5; Fraley *et al.*, *Crit. Rev. Plant Sci.* 4:1-46; and An *et al.* (1985) *EMBO J.* 4:277-287.

Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin, or chloramphenicol, *inter alia*. The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, biolistics (microparticle bombardment), or electroporation as well as other possible methods. If *Agrobacteria* are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the *vir* region

in the art. These procedures are described, for example, in Merryweather *et al.* (Merryweather, A.T., U. Weyer, M.P.G. Harris, M. Hirst, T. Booth, R.D. Possee (1990) *J. Gen. Virol.* 71:1535-1544) and Martens *et al.* (Martens, J.W.M., G. Honee, D. Zuidema, J.W.M. van Lent, B. Visser, J.M. Vlak (1990) *Appl. Environmental Microbiol.* 56(9):2764-2770).

5

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

Applicant Name(s): MYCOGEN CORPORATION
Street address: 5501 Oberlin Drive
City: San Diego
State/Province: California
Country: US
Postal code/Zip: 92121
Phone number: (619) 453-8030 Fax number: (619) 453-6991

(ii) TITLE OF INVENTION: Pesticidal Toxins

(iii) NUMBER OF SEQUENCES: 45

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Saliwanchik, Lloyd & Saliwanchik
(B) STREET: 2421 N.W. 41st Street, Suite A-1
(C) CITY: Gainesville
(D) STATE: FL
(E) COUNTRY: USA
(F) ZIP: 32606-6669

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/633,993
(B) FILING DATE: 19-APR-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Sanders, Jay M.
(B) REGISTRATION NUMBER: 39,355
(C) REFERENCE/DOCKET NUMBER: MA-703C1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 352-375-8100
(B) TELEFAX: 352-372-5800

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Leu Asp Thr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Leu Asp Thr Asn Lys Val Tyr Glu Ile Ser Asn Leu Ala Asn Gly
1 5 10 15
Leu Tyr Thr Ser Thr Tyr Leu Ser Leu
20 25

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Ala Arg Glu Val His Ile Glu Ile Asn Asn Thr Arg His Thr Leu
1 5 10 15
Gln Leu Glu Ala Lys Thr Lys Leu
20

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Leu Asp Thr Asn Lys Val Tyr Glu Ile Ser Asn His Ala Asn Gly
1           5           10           15
Leu Tyr Ala Ala Thr Tyr Leu Ser Leu
           20           25

```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 50 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Ser Ala Arg Glu Val His Ile Asp Val Asn Asn Lys Thr Gly His Thr
1           5           10           15
Leu Gln Leu Glu Asp Lys Thr Lys Leu Asp Gly Gly Arg Trp Arg Thr
           20           25           30
Ser Pro Xaa Asn Val Ala Asn Asp Gln Ile Lys Thr Phe Val Ala Glu
           35           40           45
Ser Asn
           50

```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Leu Asp Thr Asn Lys Ile Tyr Glu Ile Ser Asn Tyr Ala Asn Gly
1           5           10           15
Leu His Ala Ala Thr Tyr Leu Ser Leu
           20           25

```

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Ala Arg Glu Val His Ile Asp Val Asn Asn Lys Thr Gly His Thr
1 5 10 15
Leu Gln Leu Glu Asp Lys Thr Lys Leu
 20 25

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGNTNGATA CNAATAAAGT NTATGAAAT

29

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGATTATCTA TCTCTGAGTG TTCTTG

26

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1158 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATGTTAGATA CTAATAAAGT TTATGAAATA AGCAATCTTG CTAATGGATT ATATACATCA	60
ACTTATTTAA GTCTTGATGA TTCAGGTGTT AGTTTAAATGA GTAAAAAGGA TGAAGATATT	120
GATGATTACA ATTTAAAATG GTTTTATTT CCTATTGATA ATAATCAATA TATTATTACA	180
AGCTATGGAG CTAATAATTG TAAAGTTTGG AATGTTAAAA ATGATAAAAT AAATGTTTCA	240
ACTTATTCTT CAACAAACTC TGTACAAAAA TGGCAAATAA AAGCTAAAGA TTCTTCATAT	300
ATAATACAAA GTGATAATGG AAAGTCTTA ACAGCAGGAG TAGGTCAATC TCTTGAATA	360
GTACGCCTAA CTGATGAATT TCCAGAGAAT TCTAACCAAC AATGGAATTT AACTCCTGTA	420
CAACAATTC AACTCCCACA AAAACCTAAA ATAGATGAAA AATTAAAAGA TCATCCTGAA	480
TATTCAGAAA CCGGAAATAT AAATCCTAAA ACAACTCCTC AATTAATGGG ATGGACATTA	540
GTACCTTGTA TTATGGTAAA TGATTCAAAA ATAGATAAAA ACACTCAAAT TAAACTACT	600
CCATATTATA TTTTAAAAA ATATAAATAC TGGAATCTAG CAAAAGGAAG TAATGTATCT	660
TTACTTCCAC ATCAAAAAAG ATCATATGAT TATGAATGGG GTACAGAAAA AAATCAAAAA	720
ACAACTATTA TTAATACAGT AGGATTGCAA ATTAATATAG ATTCAGGAAT GAAATTTGAA	780
GTACCAGAAG TAGGAGGAGG TACAGAAGAC ATAAAAACAC AATTAAGTGA AGAATTAAAA	840
GTTGAATATA GCACTGAAAC CAAAATAATG ACGAAATATC AAGAACACTC AGAGATAGAT	900
AATCCAATA ATCAACCAAT GAATTCTATA GGACTTCTTA TTTATACTTC TTTAGAATTA	960
TATCGATATA ACGGTACAGA AATTAAGATA ATGGACATAG AAATTCAGA TCATGATACT	1020
TAACTCTTA CTTCTTATCC AAATCATAAA GAAGCATTAT TACTTCTCAC AAACCATTCG	1080
TATGAAGAAG TAGAAGAAAT AACAAAAATA CCTAAGCATA CACTTATAAA ATTGAAAAAA	1140
CATTATTTTA AAAAATAA	1158

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 385 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met	Leu	Asp	Thr	Asn	Lys	Val	Tyr	Glu	Ile	Ser	Asn	Leu	Ala	Asn	Gly
1				5					10					15	

Leu Tyr Thr Ser Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu
 20 25 30
 Met Ser Lys Lys Asp Glu Asp Ile Asp Asp Tyr Asn Leu Lys Trp Phe
 35 40 45
 Leu Phe Pro Ile Asp Asn Asn Gln Tyr Ile Ile Thr Ser Tyr Gly Ala
 50 55 60
 Asn Asn Cys Lys Val Trp Asn Val Lys Asn Asp Lys Ile Asn Val Ser
 65 70 75 80
 Thr Tyr Ser Ser Thr Asn Ser Val Gln Lys Trp Gln Ile Lys Ala Lys
 85 90 95
 Asp Ser Ser Tyr Ile Ile Gln Ser Asp Asn Gly Lys Val Leu Thr Ala
 100 105 110
 Gly Val Gly Gln Ser Leu Gly Ile Val Arg Leu Thr Asp Glu Phe Pro
 115 120 125
 Glu Asn Ser Asn Gln Gln Trp Asn Leu Thr Pro Val Gln Thr Ile Gln
 130 135 140
 Leu Pro Gln Lys Pro Lys Ile Asp Glu Lys Leu Lys Asp His Pro Glu
 145 150 155 160
 Tyr Ser Glu Thr Gly Asn Ile Asn Pro Lys Thr Thr Pro Gln Leu Met
 165 170 175
 Gly Trp Thr Leu Val Pro Cys Ile Met Val Asn Asp Ser Lys Ile Asp
 180 185 190
 Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Phe Lys Lys Tyr
 195 200 205
 Lys Tyr Trp Asn Leu Ala Lys Gly Ser Asn Val Ser Leu Leu Pro His
 210 215 220
 Gln Lys Arg Ser Tyr Asp Tyr Glu Trp Gly Thr Glu Lys Asn Gln Lys
 225 230 235 240
 Thr Thr Ile Ile Asn Thr Val Gly Leu Gln Ile Asn Ile Asp Ser Gly
 245 250 255
 Met Lys Phe Glu Val Pro Glu Val Gly Gly Gly Thr Glu Asp Ile Lys
 260 265 270
 Thr Gln Leu Thr Glu Glu Leu Lys Val Glu Tyr Ser Thr Glu Thr Lys
 275 280 285
 Ile Met Thr Lys Tyr Gln Glu His Ser Glu Ile Asp Asn Pro Thr Asn
 290 295 300
 Gln Pro Met Asn Ser Ile Gly Leu Leu Ile Tyr Thr Ser Leu Glu Leu
 305 310 315 320

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 834 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGACTATATG CAGCAACTTA TTTAAGTTTA GATGATTCAG GTGTTAGTTT AATGAATAAA	60
AATGATGATG ATATTGATGA TTATAACTTA AAATGGTTTT TATTTCTTAT TGATGATGAT	120
CAATATATTA TTACAAGCTA TGCAGCAAAT AATTGTAAAG TTTGGAATGT TAATAATGAT	180
AAAATAAAATG TTTCGACTTA TTCTTCAACA AATTCAATAC AAAAATGGCA AATAAAAGCT	240
AATGGTTCTT CATATGTAAT ACAAAGTGAT AATGGAAAAG TCTTAACAGC AGGAACCGGT	300
CAAGCTCTTG GATTGATACG TTAACTGAT GAATCCTCAA ATAATCCCAA TCAACAATGG	360
AATTTAACTT CTGTACAAAC AATTCAACTT CCACAAAAAC CTATAATAGA TACAAAATTA	420
AAAGATTATC CCAAATATTC ACCAACTGGA AATATAGATA ATGGAACATC TCCTCAATTA	480
ATGGGATGGA CATTAGTACC TTGTATTATG GTAAATGATC CAAATATAGA TAAAAATACT	540
CAAATTAAAA CTA CTACTCCATA TTATATTTTA AAAAAATATC AATATTGGCA ACGAGCAGTA	600
GGAAGTAATG TAGCTTTACG TCCACATGAA AAAAAATCAT ATACTTATGA ATGGGGCACA	660
GAAATAGATC AAAAAACAAC AATTATAAAT ACATTAGGAT TTCAAATCAA TATAGATTCA	720
GGAATGAAAT TTGATATACC AGAAGTAGGT GGAGGTACAG ATGAAATAAA AACACAAC TA	780
AATGAAGAAT TAAAAATAGA ATATAGTCAT GAACTAAAA TAATGGAAAA ATAT	834

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 278 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gly Leu Tyr Ala Ala Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser
 1 5 10 15
 Leu Met Asn Lys Asn Asp Asp Asp Ile Asp Asp Tyr Asn Leu Lys Trp
 20 25 30
 Phe Leu Phe Pro Ile Asp Asp Asp Gln Tyr Ile Ile Thr Ser Tyr Ala
 35 40 45
 Ala Asn Asn Cys Lys Val Trp Asn Val Asn Asn Asp Lys Ile Asn Val
 50 55 60
 Ser Thr Tyr Ser Ser Thr Asn Ser Ile Gln Lys Trp Gln Ile Lys Ala
 65 70 75 80
 Asn Gly Ser Ser Tyr Val Ile Gln Ser Asp Asn Gly Lys Val Leu Thr
 85 90 95
 Ala Gly Thr Gly Gln Ala Leu Gly Leu Ile Arg Leu Thr Asp Glu Ser
 100 105 110
 Ser Asn Asn Pro Asn Gln Gln Trp Asn Leu Thr Ser Val Gln Thr Ile
 115 120 125
 Gln Leu Pro Gln Lys Pro Ile Ile Asp Thr Lys Leu Lys Asp Tyr Pro
 130 135 140
 Lys Tyr Ser Pro Thr Gly Asn Ile Asp Asn Gly Thr Ser Pro Gln Leu
 145 150 155 160
 Met Gly Trp Thr Leu Val Pro Cys Ile Met Val Asn Asp Pro Asn Ile
 165 170 175
 Asp Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Leu Lys Lys
 180 185 190
 Tyr Gln Tyr Trp Gln Arg Ala Val Gly Ser Asn Val Ala Leu Arg Pro
 195 200 205
 His Glu Lys Lys Ser Tyr Thr Tyr Glu Trp Gly Thr Glu Ile Asp Gln
 210 215 220
 Lys Thr Thr Ile Ile Asn Thr Leu Gly Phe Gln Ile Asn Ile Asp Ser
 225 230 235 240

Gly Met Lys Phe Asp Ile Pro Glu Val Gly Gly Gly Thr Asp Glu Ile
 245 250 255

Lys Thr Gln Leu Asn Glu Glu Leu Lys Ile Glu Tyr Ser His Glu Thr
 260 265 270

Lys Ile Met Glu Lys Tyr
 275

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 829 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACATGCAGCA ACTTATTTAA GTTTAGATGA TTCAGGTGTT AGTTTAATGA ATAAAAATGA 60
 TGATGATATT GATGACTATA ATTTAAGGTG GTTTTTATTT CCTATTGATG ATAATCAATA 120
 TATTATTACA AGCTACGCAG CGAATAATTG TAAGGTTTGG AATGTTAATA ATGATAAAAT 180
 AAATGTTTCA ACTTATTCTT CAACAACTC GATACAGAAA TGGCAAATAA AAGCTAATGC 240
 TTCTTCGTAT GTAATACAAA GTAATAATGG GAAAGTTCTA ACAGCAGGAA CCGGTCAATC 300
 TCTTGGATTA ATACGTTTAA CGGATGAATC ACCAGATAAT CCCAATCAAC AATGGAATTT 360
 AACTCCTGTA CAAACAATTC AACTCCCACC AAAACCTACA ATAGATACAA AGTTAAAAGA 420
 TTACCCCAAA TATTCACAAA CTGGCAATAT AGACAAGGGA ACACCTCCTC AATTAATGGG 480
 ATGGACATTA ATACCTTGTA TTATGGTAAA TGATCCCAAT ATAGATAAAA AACTCAAAT 540
 CAAAACACT CCATATTATA TTTTAAAAAA ATATCAATAT TGGCAACAAG CAGTAGGAAG 600
 TAATGTAGCT TTACGTCCGC ATGAAAAAAA ATCATATGCT TATGAGTGGG GTACAGAAAT 660
 AGATCAAAAA ACAACTATCA TTAATACATT AGGATTTTCAG ATTAATATAG ATTCGGAAT 720
 GAAATTTGAT ATACCAGAAG TAGGTGGAGG TACAGATGAA ATAAAAACAC AATTAAACGA 780
 AGAATTAAAA ATAGAATATA GCCGTGAAAC CAAAATAATG GAAAAATAT 829

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 276 amino acids

WO 97/40162

46

- (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```

His Ala Ala Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu Met
1      5      10      15
Asn Lys Asn Asp Asp Asp Ile Asp Asp Tyr Asn Leu Arg Trp Phe Leu
20      25      30
Phe Pro Ile Asp Asp Asn Gln Tyr Ile Ile Thr Ser Tyr Ala Ala Asn
35      40      45
Asn Cys Lys Val Trp Asn Val Asn Asn Asp Lys Ile Asn Val Ser Thr
50      55      60
Tyr Ser Ser Thr Asn Ser Ile Gln Lys Trp Gln Ile Lys Ala Asn Ala
65      70      75      80
Ser Ser Tyr Val Ile Gln Ser Asn Asn Gly Lys Val Leu Thr Ala Gly
85      90      95
Thr Gly Gln Ser Leu Gly Leu Ile Arg Leu Thr Asp Glu Ser Pro Asp
100     105     110
Asn Pro Asn Gln Gln Trp Asn Leu Thr Pro Val Gln Thr Ile Gln Leu
115     120     125
Pro Pro Lys Pro Thr Ile Asp Thr Lys Leu Lys Asp Tyr Pro Lys Tyr
130     135     140
Ser Gln Thr Gly Asn Ile Asp Lys Gly Thr Pro Pro Gln Leu Met Gly
145     150     155     160
Trp Thr Leu Ile Pro Cys Ile Met Val Asn Asp Pro Asn Ile Asp Lys
165     170     175
Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Leu Lys Lys Tyr Gln
180     185     190
Tyr Trp Gln Gln Ala Val Gly Ser Asn Val Ala Leu Arg Pro His Glu
195     200     205
Lys Lys Ser Tyr Ala Tyr Glu Trp Gly Thr Glu Ile Asp Gln Lys Thr
210     215     220
Thr Ile Ile Asn Thr Leu Gly Phe Gln Ile Asn Ile Asp Ser Gly Met
225     230     235     240
Lys Phe Asp Ile Pro Glu Val Gly Gly Gly Thr Asp Glu Ile Lys Thr
245     250     255

```

Gln Leu Asn Glu Glu Leu Lys Ile Glu Tyr Ser Arg Glu Thr Lys Ile
260 265 270

Met Glu Lys Tyr
275

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ile Asp Asp Tyr Asn Leu
1 5

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Trp Phe Leu Phe Pro Ile Asp
1 5

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gln Ile Lys Thr Thr Pro Tyr Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Tyr Glu Trp Gly Thr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GATATNGATG ANTAYAAATT N

21

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGGTTTTTNT TTCCNATNGA N

21

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CAAATNAAAA CNACCCATA TTAT

24

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (synthetic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TANGANTGGG GNACAGAA

18

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (synthetic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATAATATGGN GTNGTTTTNA TTTG

24

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (synthetic)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTCTGTNCCC CANTCNTA

18

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTCAAAGCGG ATCAGGAG

18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCGTATTTCGG ATATGCTTGG

20

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 386 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
1				5						10							15
Xaa	Xaa	Xaa	Xaa	Thr	Tyr	Leu	Ser	Leu	Asp	Asp	Ser	Gly	Val	Ser	Leu		
				20				25					30				
Met	Xaa	Lys	Xaa	Asp	Xaa	Asp	Ile	Asp	Asp	Tyr	Asn	Leu	Xaa	Trp	Phe		
		35					40					45					
Leu	Phe	Pro	Ile	Asp	Xaa	Xaa	Gln	Tyr	Ile	Ile	Thr	Ser	Tyr	Xaa	Ala		
		50					55					60					
Asn	Asn	Cys	Lys	Val	Trp	Asn	Val	Xaa	Asn	Asp	Lys	Ile	Asn	Val	Ser		
65				70						75					80		
Thr	Tyr	Ser	Ser	Thr	Asn	Ser	Xaa	Gln	Lys	Trp	Gln	Ile	Lys	Ala	Xaa		
				85				90						95			
Xaa	Ser	Ser	Tyr	Xaa	Ile	Gln	Ser	Xaa	Asn	Gly	Lys	Val	Leu	Thr	Ala		
				100				105						110			
Gly	Xaa	Gly	Gln	Xaa	Leu	Gly	Xaa	Xaa	Arg	Leu	Thr	Asp	Glu	Xaa	Xaa		
		115					120					125					

Xaa Asn Xaa Asn Gln Gln Trp Asn Leu Thr Xaa Val Gln Thr Ile Gln
 130 135 140
 Leu Pro Xaa Lys Pro Xaa Ile Asp Xaa Lys Leu Lys Asp Xaa Pro Xaa
 145 150 155 160
 Tyr Ser Xaa Thr Gly Asn Ile Xaa Xaa Xaa Thr Xaa Pro Gln Leu Met
 165 170 175
 Gly Trp Thr Leu Xaa Pro Cys Ile Met Val Asn Asp Xaa Xaa Ile Asp
 180 185 190
 Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Xaa Lys Lys Tyr
 195 200 205
 Xaa Tyr Trp Xaa Xaa Ala Xaa Gly Ser Asn Val Xaa Leu Xaa Pro His
 210 215 220
 Xaa Lys Xaa Ser Tyr Xaa Tyr Glu Trp Gly Thr Glu Xaa Xaa Gln Lys
 225 230 235 240
 Thr Thr Ile Ile Asn Thr Xaa Gly Xaa Gln Ile Asn Ile Asp Ser Gly
 245 250 255
 Met Lys Phe Xaa Xaa Pro Glu Val Gly Gly Gly Thr Xaa Xaa Ile Lys
 260 265 270
 Thr Gln Leu Xaa Glu Glu Leu Lys Xaa Glu Tyr Ser Xaa Glu Thr Lys
 275 280 285
 Ile Met Xaa Lys Tyr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 290 295 300
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 305 310 315 320
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 325 330 335
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 340 345 350
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 355 360 365
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 370 375 380
 Xaa Xaa
 385

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GNGAAGTNCA TATNGAAATN AATAATAC

28

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2015 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ATTAATTTTA TGGAGGTTGA TATTTATGTC AGCTCGCGAA GTACACATTG AAATAAACAA	60
TAAAACACGT CATACATTAC AATTAGAGGA TAAAACTAAA CTTAGCGGCG GTAGATGGCG	120
AACATCACCT ACAAATGTTG CTCGTGATAC AATTAAAACA TTTGTAGCAG AATCACATGG	180
TTTTATGACA GGAGTAGAAG GTATTATATA TTTAGTGTA AACGGAGACG CAGAAATTAG	240
TTTACATTTT GACAATCCTT ATATAGGTTT TAATAAATGT GATGGTTCTT CTGATAAACC	300
TGAATATGAA GTTATTACTC AAAGCGGATC AGGAGATAAA TCTCATGTGA CATATACTAT	360
TCAGACAGTA TCTTTACGAT TATAAGGAAA ATTTATAAAA ACTGTATTTT TTAATAAAAT	420
ACCAAAAAAT ACATATTTAT TTTTGGTAT TTTCTAATAT GAAATATGAA TTATAAAAAAT	480
ATTAATAAAA AAGGTGATAA AAATTATGTT AGATACTAAT AAAGTTTATG AAATAAGCAA	540
TCTTGCTAAT GGATTATATA CATCAACTTA TTAAAGTCTT GATGATTCAG GTGTTAGTTT	600
AATGAGTAAA AAGGATGAAG ATATTGATGA TTACAATTTA AAATGGTTTT TATTTCTTAT	660
TGATAATAAT CAATATATTA TTACAAGCTA TGGAGCTAAT AATTGTAAAG TTTGGAATGT	720
TAAAAATGAT AAAATAAATG TTTCAACTTA TTCTTCAACA AACTCTGTAC AAAAATGGCA	780
AATAAAAGCT AAAGATTCTT CATATATAAT ACAAAGTGAT AATGGAAAGG TCTTAACAGC	840
AGGAGTAGGT CAATCTCTTG GAATAGTACG CCTAACTGAT GAATTTCCAG AGAATTCTAA	900
CCAACAATGG AATTAACTC CTGTACAAAC AATTCAACTC CCACAAAAAC CTAAAATAGA	960
TGAAAAATTA AAAGATCATC CTGAATATTC AGAAACCGGA AATATAAATC CTAAAACAAC	1020
TCCTCAATTA ATGGGATGGA CATTAGTACC TTGTATTATG GTAAATGATT CAAAAATAGA	1080

TAAAAACACT CAAATTA AAAA CTACTCCATA TTATATTTTT AAAAAATATA AATACTGGAA 1140
 TCTAGCAAAA GGAAGTAATG TATCTTTACT TCCACATCAA AAAAGATCAT ATGATTATGA 1200
 ATGGGGTACA GAAAAAATC AAAAAACAAC TATTATTAAT ACAGTAGGAT TGCAAATTAA 1260
 TATAGATTCA GGAATGAAAT TTGAAGTACC AGAAGTAGGA GGAGGTACAG AAGACATAAA 1320
 AACACAATTA ACTGAAGAAT TAAAAGTTGA ATATAGCACT GAAACCAAAA TAATGACGAA 1380
 ATATCAAGAA CACTCAGAGA TAGATAATCC AACTAATCAA CCAATGAATT CTATAGGACT 1440
 TCTTATTTAT ACTTCTTTAG AATTATATCG ATATAACGGT ACAGAAATTA AGATAATGGA 1500
 CATAGAACT TCAGATCATG ATACTTACAC TCTTACTTCT TATCCAAATC ATAAAGAAGC 1560
 ATTATTACTT CTCACAAACC ATTCGTATGA AGAAGTAGAA GAAATAACAA AAATACCTAA 1620
 GCATACACTT ATAAATTTGA AAAAACATTA TTTTAAAAA TAAAAACAT AATATATAAA 1680
 TGACTGATTA ATATCTCTCG AAAAGGTTCT GGTGCAAAA TAGTGGGATA TGAAAAAGC 1740
 AAAAGATTCC TAACGGAATG GAACATTAGG CTGTAAATC AAAAAGTTTA TTGATAAAAT 1800
 ATATCTGCCT TTGGACAGAC TTCTCCCCTT GGAGAGTTTG TCCTTTTTTG ACCATATGCA 1860
 TAGCTTCTAT TCCGGCAATC ATTTTGTAG CTGTTTGCAA GGATTTTAAT CCAAGCATAT 1920
 CCGAATACGC TTTTGTATAA CCGATGTCTT GTTCAATGAT ATTGTTTAAT ATTTTCACAC 1980
 GAATTGGCTA CTGTGCGGTA TCCTGTCTCC TTTAT 2015

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 360 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

ATGTCAGCTC GCGAAGTACA CATTGAAATA AACAATAAAA CACGTCATAC ATTACAATTA 60
 GAGGATAAAA CTAACTTAG CGGCGGTAGA TGGCGAACAT CACCTACAAA TGTGCTCGT 120
 GATACAATTA AAACATTTGT AGCAGAATCA CATGGTTTTA TGACAGGAGT AGAAGGTATT 180
 ATATATTTTA GTGTAAACGG AGACGCAGAA ATTAGTTTAC ATTTTGACAA TCCTTATATA 240
 GGTCTAATA AATGTGATGG TTCTTCTGAT AAACCTGAAT ATGAAGTTAT TACTCAAAGC 300
 GGATCAGGAG ATAAATCTCA TGTGACATAT ACTATTCAGA CAGTATCTTT ACGATTATAA 360

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```

Met Ser Ala Arg Glu Val His Ile Glu Ile Asn Asn Lys Thr Arg His
 1             5             10             15
Thr Leu Gln Leu Glu Asp Lys Thr Lys Leu Ser Gly Gly Arg Trp Arg
          20             25             30
Thr Ser Pro Thr Asn Val Ala Arg Asp Thr Ile Lys Thr Phe Val Ala
          35             40             45
Glu Ser His Gly Phe Met Thr Gly Val Glu Gly Ile Ile Tyr Phe Ser
          50             55             60
Val Asn Gly Asp Ala Glu Ile Ser Leu His Phe Asp Asn Pro Tyr Ile
          65             70             75             80
Gly Ser Asn Lys Cys Asp Gly Ser Ser Asp Lys Pro Glu Tyr Glu Val
          85             90             95
Ile Thr Gln Ser Gly Ser Gly Asp Lys Ser His Val Thr Tyr Thr Ile
          100            105            110
Gln Thr Val Ser Leu Arg Leu
          115

```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CATGAGATTT ATCTCCTGAT CCGC

24

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2230 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACTATGACAA TGATTATGAC TGCTGATGAA TTAGCTTTAT CAATACCAGG ATATTCTAAA	60
CCATCAAATA TAACAGGAGA TAAAAGTAAA CATACATTAT TTAATAATAT AATTGGAGAT	120
ATTCAAATAA AAGATCAAGC AACATTTGGG GTTGTTTTTG ATCCCCCTCT TAATCGTATT	180
TCAGGGGCTG AAGAATCAAG TAAGTTTATT GATGTATATT ATCCTTCTGA AGATAGTAAC	240
CTTAAATATT ATCAATTTAT AAAAGTAGCA ATTGATTTTG ATATTAATGA AGATTTTATT	300
AATTTTAATA ATCATGACAA TATAGGGATA TTTAATTTTG TTACACGAAA TTTTTTATTA	360
AATAATGAAA ATGATTAATA AAAAATTTAA TTTGTATAAT ATGTTTATTT TTTGAAAATT	420
GAATGCATAT ATTAATCGAG TATGTGTAAT AAATTTTAAT TTTATGGAGG TTGATATTTA	480
TGTCAGCACG TGAAGTACAC ATTGATGTAA ATAATAAGAC AGGTCATACA TTACAATTAG	540
AAGATAAAAC AAAACTTGAT GGTGGTAGAT GGCGAACATC ACCTACAAAT GTTGCTAATG	600
ATCAAATTAA AACATTTGTA GCAGAATCAC ATGGTTTTAT GACAGGTACA GAAGGTACTA	660
TATATTATAG TATAAATGGA GAAGCAGAAA TTAGTTTATA TTTTGACAAT CCTTATTCAG	720
GTTCTAATAA ATATGATGGG CATTCCAATA AAAATCAATA TGAAGTTATT ACCCAAGGAG	780
GATCAGGAAA TCAATCTCAT GTTACGTATA CTATTCAAAC TGTATCTTCA CGATATGGGA	840
ATAATTCATA AAAAAATATT TTTTTTACG AAAATACCAA AAAAATTTTT TTGGTATTTT	900
CTAATATAAT TCATAAATAT TTTAATAATA AAATTATAAG AAAAGGTGAT AAATATTATG	960
TTAGATACTA ATAAAATTTA TGAAATAAGT AATTATGCTA ATGGATTACA TGCAGCAACT	1020
TATTTAAGTT TAGATGATTG AGGTGTTAGT TTAATGAATA AAAATGATGA TGATATTGAT	1080
GACTATAATT TAAGGTGGTT TTTATTTCTT ATTGATGATA ATCAATATAT TATTACAAGC	1140
TACGCAGCGA ATAATTGTAA GGTGTTGAAT GTTAATAATG ATAAAATAAA TGTTTCAACT	1200
TATTCCTTCAA CAACTCGAT ACAGAAATGG CAAATAAAAG CTAATGCTTC TTCGTATGTA	1260
ATACAAAGTA ATAATGGGAA AGTTCTAACA GCAGGAACCG GTCAATCTCT TGGATTAATA	1320
CGTTTAAACG ATGAATCACC AGATAATCCC AATCAACAAT GGAATTTAAC TCCTGTACAA	1380
ACAATTCAAC TCCCACCAAA ACCTACAATA GATACAAAGT TAAAAGATTA CCCCAAATAT	1440
TCACAAACTG GCAATATAGA CAAGGGAACA CCTCCTCAAT TAATGGGATG GACATTAATA	1500

CCTTGTATTA TGGTAAATGA TCCAAATATA GATAAAAACA CTCAAATCAA AACTACTCCA	1560
TATTATATTT TAAAAAATA TCAATATTGG CAACAAGCAG TAGGAAGTAA TGTAGCTTTA	1620
CGTCCGCATG AAAAAAATC ATATGCTTAT GAGTGGGGTA CAGAAATAGA TCAAAAAACA	1680
ACTATCATT ATACATTAGG ATTTGAGATT AATATAGATT CGGGAATGAA ATTTGATATA	1740
CCAGAAGTAG GTGGAGGTAC AGATGAAATA AAAACACAAT TAAACGAAGA ATTAAAAATA	1800
GAATATAGCC GTGAAACCAA AATAATGGAA AAATATCAGG AACAATCAGA GATAGATAAT	1860
CCAACTGATC AATCAATGAA TTCTATAGGA TTCCTCACTA TTACTTCTTT AGAATTATAT	1920
CGATATAATG GTTCGGAAAT TAGTGTAATG AAAATTCAAA CTTCAGATAA TGATACTTAC	1980
AATGTGACCT CTTATCCAGA TCATCAACAA GCTCTATTAC TTCTTACAAA TCATTTCATAT	2040
GAAGAAGTAG AAGAAATAAC AAATATTCCC AAAATATCAC TGAAAAAATT AAAAAAATAT	2100
TATTTTAA ACATAATTAT ATTTGATAG CTTTTTAAAA ATAAAGATTG TTCAAAGTAA	2160
AATGAAAGAA AATCTTTTAT GAACTTTAA TACAATAAAA GAGGAATATT TTCTTATAAG	2220
TACTTCCTTG	2230

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 372 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATGTCAGCAC GTGAAGTACA CATTGATGTA AATAATAAGA CAGGTCATAC ATTACAATTA	60
GAAGATAAAA CAAAACTTGA TGGTGGTAGA TGGCGAACAT CACCTACAAA TGTTGCTAAT	120
GATCAAATTA AAACATTTGT AGCAGAATCA CATGGTTTTA TGACAGGTAC AGAAGGTACT	180
ATATATTATA GTATAAATGG AGAAGCAGAA ATTAGTTTAT ATTTTGACAA TCCTTATTCA	240
GGTTCTAATA AATATGATGG GCATTCCAAT AAAAATCAAT ATGAAGTTAT TACCCAAGGA	300
GGATCAGGAA ATCAATCTCA TGTTACGTAT ACTATTCAAA CTGTATCTTC ACGATATGGG	360
AATAATTCAT AA	372

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```

Met Ser Ala Arg Glu Val His Ile Asp Val Asn Asn Lys Thr Gly His
 1             5             10             15
Thr Leu Gln Leu Glu Asp Lys Thr Lys Leu Asp Gly Gly Arg Trp Arg
 20             25             30
Thr Ser Pro Thr Asn Val Ala Asn Asp Gln Ile Lys Thr Phe Val Ala
 35             40             45
Glu Ser His Gly Phe Met Thr Gly Thr Glu Gly Thr Ile Tyr Tyr Ser
 50             55             60
Ile Asn Gly Glu Ala Glu Ile Ser Leu Tyr Phe Asp Asn Pro Tyr Ser
 65             70             75             80
Gly Ser Asn Lys Tyr Asp Gly His Ser Asn Lys Asn Gln Tyr Glu Val
 85             90             95
Ile Thr Gln Gly Gly Ser Gly Asn Gln Ser His Val Thr Tyr Thr Ile
100            105            110
Gln Thr Val Ser Ser Arg Tyr Gly Asn Asn Ser
115            120

```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1152 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```

ATGTTAGATA CTAATAAAAT TTATGAAATA AGTAATTATG CTAATGGATT ACATGCAGCA      60
ACTTATTTAA GTTTAGATGA TTCAGGTGTT AGTTTAATGA ATAAAAATGA TGATGATATT      120
GATGACTATA ATTTAAGGTG GTTTTATTTT CCTATTGATG ATAATCAATA TATTATTACA      180
AGCTACGCAG CGAATAATTG TAAGGTTTGG AATGTTAATA ATGATAAAAT AAATGTTTCA      240
ACTTATTCTT CAACAACTC GATACAGAAA TGGCAAATAA AAGCTAATGC TTCTTCGTAT      300
GTAATACAAA GTAATAATGG GAAAGTTCTA ACAGCAGGAA CCGGTCAATC TCTTGGATTA      360

```

ATACGTTTAA CGGATGAATC ACCAGATAAT CCCAATCAAC AATGGAATTT AACTCCTGTA 420
 CAAACAATTC AACTCCCACC AAAACCTACA ATAGATACAA AGTTAAAAGA TTACCCCAAA 480
 TATTCACAAA CTGGCAATAT AGACAAGGGA ACACCTCCTC AATTAATGGG ATGGACATTA 540
 ATACCTTGTA TTATGGTAAA TGATCCAAAT ATAGATAAAA AACTCAAAT CAAAACACT 600
 CCATATTATA TTTTAAAAAA ATATCAATAT TGGCAACAAG CAGTAGGAAG TAATGTAGCT 660
 TTACGTCCGC ATGAAAAAAA ATCATATGCT TATGAGTGGG GTACAGAAAT AGATCAAAAA 720
 ACAACTATCA TTAATACATT AGGATTTCAG ATTAATATAG ATTGCGGAAT GAAATTTGAT 780
 ATACCAGAAG TAGGTGGAGG TACAGATGAA ATAAAAACAC AATTAAACGA AGAATTAAAA 840
 ATAGAATATA GCCGTGAAAC CAAAATAATG GAAAAATATC AGGAACAATC AGAGATAGAT 900
 AATCCAACGT ATCAATCAAT GAATTCTATA GGATTCCTCA CTATTACTTC TTTAGAATTA 960
 TATCGATATA ATGGTTCGGA AATTAGTGTA ATGAAAATTC AACTTCAGA TAATGATACT 1020
 TACAATGTGA CCTCTTATCC AGATCATCAA CAAGCTCTAT TACTTCTTAC AAATCATTCA 1080
 TATGAAGAAG TAGAAGAAAT AACAAATATT CCCAAATAT CACTGAAAAA ATTAAAAAAA 1140
 TATTATTTTT AA 1152

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 383 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Leu Asp Thr Asn Lys Ile Tyr Glu Ile Ser Asn Tyr Ala Asn Gly
 1 5 10 15
 Leu His Ala Ala Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu
 20 25 30
 Met Asn Lys Asn Asp Asp Asp Ile Asp Asp Tyr Asn Leu Arg Trp Phe
 35 40 45
 Leu Phe Pro Ile Asp Asp Asn Gln Tyr Ile Ile Thr Ser Tyr Ala Ala
 50 55 60
 Asn Asn Cys Lys Val Trp Asn Val Asn Asn Asp Lys Ile Asn Val Ser
 65 70 75 80

Thr Tyr Ser Ser Thr Asn Ser Ile Gln Lys Trp Gln Ile Lys Ala Asn
 85 90 95
 Ala Ser Ser Tyr Val Ile Gln Ser Asn Asn Gly Lys Val Leu Thr Ala
 100 105 110
 Gly Thr Gly Gln Ser Leu Gly Leu Ile Arg Leu Thr Asp Glu Ser Pro
 115 120 125
 Asp Asn Pro Asn Gln Gln Trp Asn Leu Thr Pro Val Gln Thr Ile Gln
 130 135 140
 Leu Pro Pro Lys Pro Thr Ile Asp Thr Lys Leu Lys Asp Tyr Pro Lys
 145 150 155 160
 Tyr Ser Gln Thr Gly Asn Ile Asp Lys Gly Thr Pro Pro Gln Leu Met
 165 170 175
 Gly Trp Thr Leu Ile Pro Cys Ile Met Val Asn Asp Pro Asn Ile Asp
 180 185 190
 Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Leu Lys Lys Tyr
 195 200 205
 Gln Tyr Trp Gln Gln Ala Val Gly Ser Asn Val Ala Leu Arg Pro His
 210 215 220
 Glu Lys Lys Ser Tyr Ala Tyr Glu Trp Gly Thr Glu Ile Asp Gln Lys
 225 230 235 240
 Thr Thr Ile Ile Asn Thr Leu Gly Phe Gln Ile Asn Ile Asp Ser Gly
 245 250 255
 Met Lys Phe Asp Ile Pro Glu Val Gly Gly Gly Thr Asp Glu Ile Lys
 260 265 270
 Thr Gln Leu Asn Glu Glu Leu Lys Ile Glu Tyr Ser Arg Glu Thr Lys
 275 280 285
 Ile Met Glu Lys Tyr Gln Glu Gln Ser Glu Ile Asp Asn Pro Thr Asp
 290 295 300
 Gln Ser Met Asn Ser Ile Gly Phe Leu Thr Ile Thr Ser Leu Glu Leu
 305 310 315 320
 Tyr Arg Tyr Asn Gly Ser Glu Ile Ser Val Met Lys Ile Gln Thr Ser
 325 330 335
 Asp Asn Asp Thr Tyr Asn Val Thr Ser Tyr Pro Asp His Gln Gln Ala
 340 345 350
 Leu Leu Leu Leu Thr Asn His Ser Tyr Glu Glu Val Glu Glu Ile Thr
 355 360 365
 Asn Ile Pro Lys Ile Ser Leu Lys Lys Leu Lys Lys Tyr Tyr Phe
 370 375 380

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2132 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GTATTCAGG GGGTGAAGAT TCAAGTAAGT TTATTGATGT ATATTATCCT TTTGAAGATA	60
GTAAATTTTAA ATATTATCAA TTTATAAAAG TAGCAATTGA TTTTGATATT AATGAAGATT	120
TTATTAATTT TAATAATCAT GACAATATAG GGATATTTAA TTTTGTTACA CGAAATTTTT	180
TATTAAATAA TGAAATGAT GAATAAAAAA TTTAATTTGT TTATTATGTT TATTTTTTGA	240
AAATGAATG CATATATTAA TCGAGTATGT ATAATAAATT TTAATTTTAT GGAGGTTGAT	300
ATTTATGTCA GCACGTGAAG TACACATTGA TGTAATAAAT AAGACAGGTC ATACATTACA	360
ATTAGAAGAT AAAACAAAAC TTGATGGTGG TAGATGGCGA ACATCACCTA CAAATGTTGC	420
TAATGATCAA ATTAAACAT TTGTAGCAGA ATCAAATGGT TTTATGACAG GTACAGAAGG	480
TACTATATAT TATAGTATAA ATGGAGAAGC AGAAATTAGT TTATATTTTG ACAATCCTTT	540
TGCAGGTTCT AATAAATATG ATGGACATTC CAATAAATCT CAATATGAAA TTATTACCCA	600
AGGAGGATCA GGAAATCAAT CTCATGTTAC GTATACTATT CAAACCACAT CCTCAGGATA	660
TGGGCATAAA TCATAACAAA TAATTTTTTA CGAAATACC AAAAAATAAA TATTTTTTGG	720
TATTTTCTAA TATAAATTAC AAATATATTA ATAATAAAT TATAAGAAAA GGTGATAAAG	780
ATTATGTTAG ATACTAATAA AGTTTATGAA ATAAGCAATC ATGCTAATGG ACTATATGCA	840
GCAACTTATT TAAGTTTAGA TGATTCAGGT GTTAGTTTAA TGAATAAAAA TGATGATGAT	900
ATTGATGATT ATAACTTAAA ATGGTTTTTA TTCCTATTG ATGATGATCA ATATATTATT	960
ACAAGCTATG CAGCAAATAA TTGTAAAGTT TGGAATGTTA ATAATGATAA AATAAATGTT	1020
TCGACTTATT CTTCAACAAA TTCAATACAA AAATGGCAAA TAAAAGCTAA TGGTCTTCTA	1080
TATGTAATAC AAAGTGATAA TGGAAAAGTC TTAACAGCAG GAACCGGTCA AGCTCTTGGA	1140
TTGATACGTT TAACTGATGA ATCCTCAAAT AATCCCAATC AACAATGGAA TTAACTTCT	1200
GTACAAACAA TTCAACTTCC ACAAAAACCT ATAATAGATA CAAAATTAAA AGATTATCCC	1260
AAATATTCAC CAACTGGAAA TATAGATAAT GGAACATCTC CTCAATTAAT GGGATGGACA	1320
TTAGTACCTT GTATTATGGT AAATGATCCA AATATAGATA AAAATACTCA AATTAAAACT	1380

ACTCCATATT ATATTTTAAA AAAATATCAA TATTGGCAAC GAGCAGTAGG AAGTAATGTA 1440
 GCTTTACGTC CACATGAAAA AAAATCATAT ACTTATGAAT GGGGCACAGA AATAGATCAA 1500
 AAAACAACAA TTATAAATAC ATTAGGATTT CAAATCAATA TAGATTCAGG AATGAAATTT 1560
 GATATACCAG AAGTAGGTGG AGGTACAGAT GAAATAAAAA CACAACTAAA TGAAGAATTA 1620
 AAAATAGAAT ATAGTCATGA AACTAAAATA ATGGAAAAAT ATCAAGAACA ATCTGAAATA 1680
 GATAATCCAA CTGATCAATC AATGAATTCT ATAGGATTTC TTACTATTAC TTCCTTAGAA 1740
 TTATATAGAT ATAATGGCTC AGAAATTCGT ATAATGCAAA TTCAAACCTC AGATAATGAT 1800
 ACTTATAATG TTACTTCTTA TCCAAATCAT CAACAAGCTT TATTACTTCT TACAAATCAT 1860
 TCATATGAAG AAGTAGAAGA AATAACAAAT ATTCTTAAAA GTACACTAAA AAAATTAAAA 1920
 AAATATTATT TTAAATATT GAAATTAGAA ATTATCTAAA ACAAACGAA AGATAATTTA 1980
 ATCTTTAATT ATTTGTAAGA TAATCGTATT TTATTTGTAT TAATTTTAT ACAATATAAA 2040
 GTAATATCTG TACGTGAAAT TGGTTTCGCT TCAATATCTA ATCTCATCTC ATGTATTACA 2100
 TGCCTAATAC CTCTTGTTTC TGCTTCTACA AG 2132

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 372 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ATGTCAGCAC GTGAAGTACA CATTGATGTA AATAATAAGA CAGGTCATAC ATTACAATTA 60
 GAAGATAAAA CAAAACTGA TGGTGGTAGA TGGCGAACAT CACCTACAAA TGTTGCTAAT 120
 GATCAAATTA AAACATTGT AGCAGAATCA AATGGTTTTA TGACAGGTAC AGAAGGTACT 180
 ATATATTATA GTATAAATGG AGAAGCAGAA ATTAGTTTAT ATTTTGACAA TCCTTTTGCA 240
 GGTCTAATA AATATGATGG ACATTCCAAT AAATCTCAAT ATGAAATTAT TACCCAAGGA 300
 GGATCAGGAA ATCAATCTCA TGTTACGTAT ACTATTCAAA CCACATCCTC ACGATATGGG 360
 CATAAATCAT AA 372

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```

Met Ser Ala Arg Glu Val His Ile Asp Val Asn Asn Lys Thr Gly His
 1             5             10             15

Thr Leu Gln Leu Glu Asp Lys Thr Lys Leu Asp Gly Gly Arg Trp Arg
 20             25             30

Thr Ser Pro Thr Asn Val Ala Asn Asp Gln Ile Lys Thr Phe Val Ala
 35             40             45

Glu Ser Asn Gly Phe Met Thr Gly Thr Glu Gly Thr Ile Tyr Tyr Ser
 50             55             60

Ile Asn Gly Glu Ala Glu Ile Ser Leu Tyr Phe Asp Asn Pro Phe Ala
 65             70             75             80

Gly Ser Asn Lys Tyr Asp Gly His Ser Asn Lys Ser Gln Tyr Glu Ile
 85             90             95

Ile Thr Gln Gly Ser Gly Asn Gln Ser His Val Thr Tyr Thr Ile
100             105             110

Gln Thr Thr Ser Ser Arg Tyr Gly His Lys Ser
115             120

```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1152 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```

ATGTTAGATA CTAATAAAGT TTATGAAATA AGCAATCATG CTAATGGACT ATATGCAGCA      60
ACTTATTTAA GTTTAGATGA TTCAGGTGTT AGTTTAATGA ATAAAAATGA TGATGATATT      120
GATGATTATA ACTTAAATG GTTTTTATTT CCTATTGATG ATGATCAATA TATTATTACA      180
AGCTATGCAG CAAATAATTG TAAAGTTTGG AATGTTAATA ATGATAAAAT AAATGTTTCG      240
ACTTATTCTT CAACAAATTC AATACAAAAA TGGCAAATAA AAGCTAATGG TTCTTCATAT      300
GTAATACAAA GTGATAATGG AAAAGTCTTA ACAGCAGGAA CCGGTCAAGC TCTTGGATTG      360

```

ATACGTTTAA CTGATGAATC CTCAAATAAT CCCAATCAAC AATGGAATTT AACTTCTGTA 420
 CAAACAATTC AACTTCCACA AAAACCTATA ATAGATACAA AATTAAAAGA TTATCCCAAA 480
 TATTCACCAA CTGGAAATAT AGATAATGGA ACATCTCCTC AATTAATGGG ATGGACATTA 540
 GTACCTTGTA TTATGGTAAA TGATCCAAAT ATAGATAAAA ATACTCAAAT TAAAACTACT 600
 CCATATTATA TTTTAAAAAA ATATCAATAT TGGCAACGAG CAGTAGGAAG TAATGTAGCT 660
 TTACGTCCAC ATGAAAAAAA ATCATATACT TATGAATGGG GCACAGAAAT AGATCAAAAA 720
 ACAACAATTA TAAATACATT AGGATTTCAA ATCAATATAG ATTCAGGAAT GAAATTTGAT 780
 ATACCAGAAG TAGGTGGAGG TACAGATGAA ATAAAAACAC AACTAAATGA AGAATTAAAA 840
 ATAGAATATA GTCATGAAAC TAAAATAATG GAAAAATATC AAGAACAATC TGAAATAGAT 900
 AATCCAACCTG ATCAATCAAT GAATTCTATA GGATTTCTTA CTATTACTTC CTTAGAATTA 960
 TATAGATATA ATGGCTCAGA AATTCGTATA ATGCAAATTC AAACCTCAGA TAATGATACT 1020
 TATAATGTTA CTTCTTATCC AAATCATCAA CAAGCTTTAT TACTTCTTAC AAATCATTCA 1080
 TATGAAGAAG TAGAAGAAAT AACAAATATT CCTAAAAGTA CACTAAAAAA ATTAAAAAAA 1140
 TATTATTTTT AA 1152

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 383 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Leu Asp Thr Asn Lys Val Tyr Glu Ile Ser Asn His Ala Asn Gly
 1 5 10 15
 Leu Tyr Ala Ala Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu
 20 25 30
 Met Asn Lys Asn Asp Asp Asp Ile Asp Asp Tyr Asn Leu Lys Trp Phe
 35 40 45
 Leu Phe Pro Ile Asp Asp Asp Gln Tyr Ile Ile Thr Ser Tyr Ala Ala
 50 55 60
 Asn Asn Cys Lys Val Trp Asn Val Asn Asn Asp Lys Ile Asn Val Ser
 65 70 75 80

Thr Tyr Ser Ser Thr Asn Ser Ile Gln Lys Trp Gln Ile Lys Ala Asn
 85 90 95
 Gly Ser Ser Tyr Val Ile Gln Ser Asp Asn Gly Lys Val Leu Thr Ala
 100 105 110
 Gly Thr Gly Gln Ala Leu Gly Leu Ile Arg Leu Thr Asp Glu Ser Ser
 115 120 125
 Asn Asn Pro Asn Gln Gln Trp Asn Leu Thr Ser Val Gln Thr Ile Gln
 130 135 140
 Leu Pro Gln Lys Pro Ile Ile Asp Thr Lys Leu Lys Asp Tyr Pro Lys
 145 150 155 160
 Tyr Ser Pro Thr Gly Asn Ile Asp Asn Gly Thr Ser Pro Gln Leu Met
 165 170 175
 Gly Trp Thr Leu Val Pro Cys Ile Met Val Asn Asp Pro Asn Ile Asp
 180 185 190
 Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Leu Lys Lys Tyr
 195 200 205
 Gln Tyr Trp Gln Arg Ala Val Gly Ser Asn Val Ala Leu Arg Pro His
 210 215 220
 Glu Lys Lys Ser Tyr Thr Tyr Glu Trp Gly Thr Glu Ile Asp Gln Lys
 225 230 235 240
 Thr Thr Ile Ile Asn Thr Leu Gly Phe Gln Ile Asn Ile Asp Ser Gly
 245 250 255
 Met Lys Phe Asp Ile Pro Glu Val Gly Gly Gly Thr Asp Glu Ile Lys
 260 265 270
 Thr Gln Leu Asn Glu Glu Leu Lys Ile Glu Tyr Ser His Glu Thr Lys
 275 280 285
 Ile Met Glu Lys Tyr Gln Glu Gln Ser Glu Ile Asp Asn Pro Thr Asp
 290 295 300
 Gln Ser Met Asn Ser Ile Gly Phe Leu Thr Ile Thr Ser Leu Glu Leu
 305 310 315 320
 Tyr Arg Tyr Asn Gly Ser Glu Ile Arg Ile Met Gln Ile Gln Thr Ser
 325 330 335
 Asp Asn Asp Thr Tyr Asn Val Thr Ser Tyr Pro Asn His Gln Gln Ala
 340 345 350
 Leu Leu Leu Leu Thr Asn His Ser Tyr Glu Glu Val Glu Glu Ile Thr
 355 360 365
 Asn Ile Pro Lys Ser Thr Leu Lys Lys Leu Lys Lys Tyr Tyr Phe
 370 375 380

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
ATGTCCGCCC GCGAGGTGCA CATCGAGATC AACACAAGA CCCGCCACAC CCTCCAGCTC      60
GAGGACAAGA CCAAGCTCTC CGGCGGCAGG TGGCGCACCT CCCCACCAA CGTGGCCCGC      120
GACACCATCA AGACGTTCTG GCGGAGTCC CACGGCTTCA TGACCGGCGT CGAGGGCATC      180
ATCTACTTCT CCGTGAACGG CGACGCCGAG ATCTCCCTCC ACTTCGACAA CCCGTACATC      240
GGCTCCAACA AGTGCGACGG CTCCTCCGAC AAGCCCGAGT ACGAGGTGAT CACCCAGTCC      300
GGCTCCGGCG ACAAGTCCCA CGTGACCTAC ACCATCCAGA CCGTGTCCCT CCGCCTCTGA      360
```

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1158 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
ATGCTCGACA CCAACAAGGT GTACGAGATC TCCAACCTCG CCAACGGCCT CTACACCTCC      60
ACCTACCTCT CCCTCGACGA CTCGCGCGTG TCCCTCATGT CCAAGAAGGA CGAGGACATC      120
GACGACTACA ACCTCAAGTG GTTCTCTTTC CCGATCGACA ACAACCAGTA CATCATCACC      180
TCCTACGGCG CCAACAACCTG CAAGGTGTGG AACGTGAAGA ACGACAAGAT CAACGTGTCC      240
ACCTACTCCT CCACCAACTC CGTGCGAAG TGGCAGATCA AGGCCAAGGA CTCCTCTTAC      300
ATCATCCAGT CCGACAACGG CAAGGTGCTC ACCCGGGCG TGGGCCAGTC CCTCGGCATC      360
GTGCGCCTCA CCGACGAGTT CCCGGAGAAC TCCAACCAGC AATGGAACCT CACCCCGGTG      420
CAGACCATCC AGCTCCCGCA GAAGCCGAAG ATCGACGAGA AGCTCAAGGA CCACCCGGAG      480
TACTCCGAGA CCGGCAACAT CAACCCGAAG ACCACCCCGC AGCTCATGGG CTGGACCCTC      540
GTGCCGTGCA TCATGGTGAA CGACTCCAAG ATCGACAAGA ACACCCAGAT CAAGACCACC      600
```

CCGTACTACA TCTTCAAGAA ATACAAGTAC TGGAACCTCG CCAAGGGCTC CAACGTGTCC	660
CTCCTCCCGC ACCAGAAGCG CAGCTACGAC TACGAGTGGG GCACCGAGAA GAACCAGAAG	720
ACCACCATCA TCAACACCGT GGGCCTGCAG ATCAACATCG ACTCGGGGAT GAAGTTCGAG	780
GTGCCGGAGG TGGGCGGCGG CACCGAGGAC ATCAAGACCC AGCTCACCGA GGAGCTGAAG	840
GTGGAGTACT CCACCGAGAC CAAGATCATG ACCAAGTACC AGGAGCACTC CGAGATCGAC	900
AACCCGACCA ACCAGCCGAT GAACTCCATC GGCCTCCTCA TCTACACCTC CCTCGAGCTG	960
TACCGCTACA ACGGCACCGA GATCAAGATC ATGGACATCG AGACCTCCGA CCACGACACC	1020
TACACCCTCA CCTCTACCC GAACCACAAG GAGGCGCTGC TGCTGCTGAC CAACCACTCC	1080
TACGAGGAGG TGGAGGAGAT CACCAAGATC CCGAAGCACA CCCTCATCAA GCTCAAGAAG	1140
CACTACTTCA AGAAGTGA	1158

Claims

1 1. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein said nucleotide sequence hybridizes under
3 stringent conditions with a nucleotide sequence selected from the group consisting of: DNA
4 which encodes SEQ ID NO. 2; DNA which encodes SEQ ID NO. 4; DNA which encodes SEQ
5 ID NO. 6; SEQ ID NO. 8; SEQ ID NO. 10; DNA which encodes SEQ ID NO. 11; SEQ ID NO.
6 12; DNA which encodes SEQ ID NO. 13; SEQ ID NO. 14; DNA which encodes SEQ ID NO.
7 15; DNA which encodes SEQ ID NO. 16; DNA which encodes SEQ ID NO. 17; DNA which
8 encodes SEQ ID NO. 18; DNA which encodes SEQ ID NO. 19; SEQ ID NO. 20; SEQ ID NO.
9 21; SEQ ID NO. 22; SEQ ID NO. 23; SEQ ID NO. 24; SEQ ID NO. 25; SEQ ID NO. 26; SEQ
10 ID NO. 27; DNA which encodes a pesticidal portion of SEQ ID NO. 28; SEQ ID NO. 37; DNA
11 which encodes SEQ ID NO. 38; SEQ ID NO. 42; and DNA which encodes SEQ ID NO. 43.

1 2. The isolated polynucleotide, according to claim 1, wherein said nucleotide sequence
2 hybridizes with DNA which encodes SEQ ID NO. 2.

1 3. The isolated polynucleotide, according to claim 1, wherein said nucleotide sequence
2 hybridizes with SEQ ID NO. 10.

1 4. The isolated polynucleotide, according to claim 1, wherein said toxin has a molecular
2 weight of approximately 40-50 kDa.

1 5. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein said toxin immunoreacts with an antibody to an
3 approximately 40-50 kDa toxin from a *Bacillus thuringiensis* isolate selected from the group
4 consisting of PS80JJ1, having the identifying characteristics of NRRL B-18679; PS149B1,
5 having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying
6 characteristics of NRRL B-21554.

1 6. The isolated polynucleotide, according to claim 5, wherein said nucleotide sequence
2 encodes a toxin of approximately 40-50 kDa.

1 7. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein a portion of said nucleotide sequence can be
3 amplified by PCR using a primer pair selected from the following group:

4 SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp; SEQ ID NOS. 20 and
5 25 to produce a fragment of about 594 bp; SEQ ID NOS. 21 and 24 to produce a fragment of
6 about 471 bp; and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp.

1 8. The isolated polynucleotide, according to claim 7, wherein said nucleotide sequence
2 encodes a toxin of approximately 40-50 kDa.

1 9. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein said toxin comprises a pesticidal portion of an
3 amino acid sequence shown in the group selected from SEQ ID NO. 30, SEQ ID NO. 34, and
4 SEQ ID NO. 39.

1 10. The isolated polynucleotide, according to claim 9, wherein said nucleotide sequence
2 encodes a toxin which comprises a pesticidal portion of the consensus sequence shown in Figure
3 1.

1 11. The isolated polynucleotide, according to claim 9, wherein said nucleotide sequence
2 encodes a toxin of approximately 40-50 kDa.

1 12. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein said toxin comprises an amino acid sequence
3 which has at least about 75% homology with a pesticidal portion of an amino acid sequence
4 selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ
5 ID NO. 38, and SEQ ID NO. 43.

1 13. The isolated polynucleotide, according to claim 12, wherein said nucleotide
2 sequence encodes a toxin which comprises an amino acid sequence which has at least about 80%
3 homology with a pesticidal portion of an amino acid sequence selected from the group consisting
4 of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

1 14. The isolated polynucleotide, according to claim 12, wherein said nucleotide
2 sequence encodes a toxin which comprises an amino acid sequence which has at least about 90%
3 homology with a pesticidal portion of an amino acid sequence selected from the group consisting
4 of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

1 15. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 which is active against a non-mammalian pest, wherein said nucleotide sequence is from a
3 *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1, having the
4 identifying characteristics of NRRL B-18679; PS149B1, having the identifying characteristics
5 of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554; and
6 mutants thereof which retain pesticidal activity.

1 16. The isolated polynucleotide, according to claim 15, wherein said toxin is
2 approximately 40-50 kDa.

1 17. The isolated polynucleotide, according to claim 15, wherein said toxin is
2 approximately 10-15 kDa.

1 18. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein said nucleotide sequence hybridizes under
3 stringent conditions with a nucleotide sequence selected from the group consisting of: DNA
4 which encodes SEQ ID NO. 3; DNA which encodes SEQ ID NO. 5; and DNA which encodes
5 SEQ ID NO. 7.

1 19. The isolated polynucleotide, according to claim 18, wherein said nucleotide
2 sequence encodes a toxin of about 10-15 kDa.

1 20. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein said toxin immunoreacts with an antibody to an
3 approximately 10-15 kDa toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
4 selected from the group consisting of PS80JJ1, having the identifying characteristics of NRRL
5 B-18679; PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2,
6 having the identifying characteristics of NRRL B-21554.

1 21. The isolated polynucleotide, according to claim 20, wherein said nucleotide
2 sequence encodes a toxin of approximately 10-15 kDa.

1 22. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein a portion of said nucleotide sequence can be
3 amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33.

1 23. The isolated polynucleotide, according to claim 22, wherein said nucleotide
2 sequence encodes a toxin of approximately 10-15 kDa.

1 24. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein said toxin comprises a pesticidal portion of an
3 amino acid sequence selected from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and
4 SEQ ID NO. 41.

1 25. The isolated polynucleotide, according to claim 24, wherein said toxin comprises
2 the amino acid sequence shown in SEQ ID NO. 32.

1 26. The isolated polynucleotide, according to claim 24, wherein said nucleotide
2 sequence encodes a toxin of approximately 10-15 kDa.

1 27. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin
2 active against a non-mammalian pest wherein said toxin comprises an amino acid sequence
3 which has at least about 75% homology with an amino acid sequence selected from the group
4 consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO.
5 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

1 28. The isolated polynucleotide, according to claim 27, wherein said nucleotide
2 sequence encodes a toxin which comprises an amino acid sequence which has at least about 80%
3 homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3,
4 SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ
5 ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

1 29. The isolated polynucleotide, according to claim 27, wherein said nucleotide
2 sequence encodes a toxin which comprises an amino acid sequence which has at least about 90%
3 homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3,
4 SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ
5 ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

1 30. A purified toxin active against a non-mammalian pest, wherein said toxin is
2 encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide
3 sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2; DNA
4 which encodes SEQ ID NO. 4; DNA which encodes SEQ ID NO. 6; SEQ ID NO. 8; SEQ ID
5 NO. 10; DNA which encodes SEQ ID NO. 11; SEQ ID NO. 12; DNA which encodes SEQ ID
6 NO. 13; SEQ ID NO. 14; DNA which encodes SEQ ID NO. 15; DNA which encodes SEQ ID
7 NO. 16; DNA which encodes SEQ ID NO. 17; DNA which encodes SEQ ID NO. 18; DNA
8 which encodes SEQ ID NO. 19; SEQ ID NO. 20; SEQ ID NO. 21; SEQ ID NO. 22; SEQ ID NO.
9 23; SEQ ID NO. 24; SEQ ID NO. 25; SEQ ID NO. 26; SEQ ID NO. 27; DNA which encodes
10 a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38,
11 SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43.

1 31. The purified toxin, according to claim 30, wherein said toxin does not have the
2 amino acid sequence shown in SEQ ID NO. 11.

1 32. The purified toxin, according to claim 31, wherein said toxin is encoded by a
2 nucleotide sequence which hybridizes with DNA which encodes SEQ ID NO. 2.

1 33. The purified toxin, according to claim 31, which is encoded by DNA which
2 hybridizes with SEQ ID NO. 10.

1 34. The purified toxin, according to claim 31, having a molecular weight of
2 approximately 40-50 kDa.

1 35. A purified toxin active against a non-mammalian pest, wherein said toxin
2 immunoreacts with an antibody to an approximately 40-50 kDa toxin, or a fragment thereof,
3 from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1, having the

identifying characteristics of NRRL B-18679; PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554.

36. The purified toxin, according to claim 35, wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

37. The purified toxin, according to claim 36, having a molecular weight of about 40-50 kDa.

38. A purified toxin having activity against a non-mammalian pest, wherein said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the following group:

SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp; SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp; SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp; and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp.

39. The purified toxin, according to claim 38, wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

40. The purified toxin, according to claim 39, having a molecular weight of about 40-50 kDa.

41. A purified toxin active against a non-mammalian pest, wherein said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28.

42. The purified toxin, according to claim 41, wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

43. The purified toxin, according to claim 42, wherein said toxin comprises a pesticidal portion of the consensus sequence in Figure 1.

44. The purified toxin, according to claim 42, having a molecular weight of approximately 40-50 kDa.

1 45. A purified toxin active against a non-mammalian pest, wherein said toxin comprises
2 an amino acid sequence which has at least about 75% homology with a pesticidal portion of an
3 amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13,
4 SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

1 46. The purified toxin, according to claim 45, wherein said toxin does not have the
2 amino acid sequence shown in SEQ ID NO. 11.

1 47. The purified toxin, according to claim 46, which comprises an amino acid sequence
2 which has at least about 80% homology with a pesticidal portion of an amino acid sequence
3 selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, and SEQ ID NO. 15,
4 SEQ ID NO. 38, and SEQ ID NO. 43.

1 48. The purified toxin, according to claim 46, which comprises an amino acid sequence
2 which has at least about 90% homology with a pesticidal portion of an amino acid sequence
3 selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ
4 ID NO. 38, and SEQ ID NO. 43.

1 49. The purified toxin, according to claim 46, having a molecular weight of
2 approximately 40-50 kDa.

1 50. A purified toxin active against a non-mammalian pest, wherein said toxin is
2 encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide
3 sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 3; DNA
4 which encodes SEQ ID NO. 5; and DNA which encodes SEQ ID NO. 7.

1 51. The purified toxin, according to claim 50, having a molecular weight of
2 approximately 10-15 kDa.

1 52. A purified toxin active against a non-mammalian pest, wherein said toxin
2 immunoreacts with an antibody to an approximately 10-15 kDa toxin, or a fragment thereof,
3 from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1, having the
4 identifying characteristics of NRRL B-18679; PS149B1, having the identifying characteristics
5 of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554.

1 53. The purified toxin, according to claim 52, having a molecular weight of about 10-15
2 kDa.

1 54. A purified toxin having activity against a non-mammalian pest, wherein said toxin
2 is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be
3 amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33.

1 55. The purified toxin, according to claim 54, having a molecular weight of about 10-15
2 kDa.

1 56. A purified toxin active against a non-mammalian pest, wherein said toxin comprises
2 a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID
3 NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41.

1 57. The purified toxin, according to claim 56, wherein said toxin comprises the amino
2 acid sequence shown in SEQ ID NO. 32.

1 58. The purified toxin, according to claim 56, having a molecular weight of
2 approximately 10-15 kDa.

1 59. A purified toxin active against a non-mammalian pest, wherein said toxin comprises
2 an amino acid sequence which has at least about 75% homology with an amino acid sequence
3 selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal
4 portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of
5 SEQ ID NO. 41.

1 60. The purified toxin, according to claim 59, which comprises an amino acid sequence
2 which has at least about 80% homology with an amino acid sequence selected from the group
3 consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO.
4 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

1 61. The purified toxin, according to claim 59, which comprises an amino acid sequence
2 which has at least about 90% homology with an amino acid sequence selected from the group

3 consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7; pesticidal portions of SEQ ID NO.
4 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

1 62. A biologically pure culture of a *Bacillus thuringiensis* isolate selected from the
2 group consisting of PS149B1, having the identifying characteristics of NRRL B-21553; and
3 PS167H2, having the identifying characteristics of NRRL B-21554; and mutants thereof which
4 retain pesticidal activity.

1 63. The biologically pure culture, according to claim 62, wherein said *Bacillus*
2 *thuringiensis* isolate is PS149B1, having the identifying characteristics of NRRL B-21553.

1 64. The biologically pure culture, according to claim 62, wherein said *Bacillus*
2 *thuringiensis* isolate is PS167H2, having the identifying characteristics of NRRL B-21554.

1 65. A composition of matter for controlling coleopterans comprising a *Bacillus*
2 *thuringiensis* isolate selected from the group consisting of PS149B1, having the identifying
3 characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL
4 B-21554; and mutants thereof which retain activity against coleopterans, in association with an
5 agricultural carrier appropriate for use in controlling coleopterans.

1 66. A method for controlling a non-mammalian pest comprising contacting said pest
2 with a pesticidal amount of a *Bacillus thuringiensis* isolate, or a toxin of said *Bacillus*
3 *thuringiensis* isolate, wherein said isolate is selected from the group consisting of PS149B1,
4 having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying
5 characteristics of NRRL B-21554; and mutants thereof which retain pesticidal activity.

1 67. The method, according to claim 66, wherein said *Bacillus thuringiensis* isolate is
2 PS149B1, having the identifying characteristics of NRRL B-21553.

1 68. The method, according to claim 66, wherein said *Bacillus thuringiensis* isolate is
2 PS167H2, having the identifying characteristics of NRRL B-21554.

1 69. A method for controlling a non-mammalian pest which comprises contacting said
2 pest with a pesticidal amount of a *Bacillus thuringiensis* toxin wherein said toxin has a
3 characteristic selected from the group consisting of:

- 4 (a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent
5 conditions with a nucleotide sequence selected from the group consisting of:
6 DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA
7 which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which
8 encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO.
9 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes
10 SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes
11 SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ
12 ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25,
13 SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of
14 SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ
15 ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin
16 does not have the amino acid sequence shown in SEQ ID NO. 11;
- 17 (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa
18 pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
19 selected from the group consisting of PS80JJ1 having the identifying
20 characteristics of NRRL B-18679, PS149B1 having the identifying
21 characteristics of NRRL B-21553, and PS167H2 having the identifying
22 characteristics of NRRL B-21554, and wherein said toxin does not have the
23 amino acid sequence shown in SEQ ID NO. 11;
- 24 (c) said toxin is encoded by a nucleotide sequence wherein a portion of said
25 nucleotide sequence can be amplified by PCR using a primer pair selected from
26 the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about
27 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ
28 ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS.
29 21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does
30 not have the amino acid sequence shown in SEQ ID NO. 11;
- 31 (d) said toxin comprises a pesticidal portion of the amino acid sequence shown in
32 SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence
33 shown in SEQ ID NO. 11;

- 34 (e) said toxin comprises an amino acid sequence which has at least about 75%
35 homology with an amino acid sequence selected from the group consisting of
36 SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ
37 ID NO. 43; and wherein said toxin does not have the amino acid sequence
38 shown in SEQ ID NO. 11;
- 39 (f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent
40 conditions with a nucleotide sequence selected from the group consisting of
41 DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and
42 DNA which encodes SEQ ID NO. 7;
- 43 (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa
44 pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
45 selected from the group consisting of PS80JJ1 having the identifying
46 characteristics of NRRL B-18679, PS149B1 having the identifying
47 characteristics of NRRL B-21553, and PS167H2 having the identifying
48 characteristics of NRRL B-21554;
- 49 (h) said toxin is encoded by a nucleotide sequence wherein a portion of said
50 nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID
51 NO. 29 and SEQ ID NO. 33;
- 52 (i) said toxin comprises a pesticidal portion of an amino acid sequence selected
53 from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO.
54 41; and
- 55 (j) said toxin comprises an amino acid sequence which has at least about 75%
56 homology with an amino acid sequence selected from the group consisting of
57 SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID
58 NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of
59 sequence IDS NO. 41.

1 70. The method, according to claim 69, wherein the full length of said toxin is
2 approximately 40-50 kDa.

1 71. The method, according to claim 69, wherein said toxin is encoded by a nucleotide
2 sequence which hybridizes under stringent conditions with a nucleotide sequence selected from
3 the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO.
4 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes

5 SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA
6 which encodes SEQ ID NO. 15, DNA which encodes SEQ ID NO. 16, DNA which encodes SEQ
7 ID NO. 17, DNA which encodes SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ
8 ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO.
9 25, SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of SEQ ID NO.
10 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which
11 encodes SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown
12 in SEQ ID NO. 11.

1 72. The method, according to claim 69, wherein said toxin immunoreacts with an
2 antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus*
3 *thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying
4 characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-
5 21553, and PS167H2 having the identifying characteristics of NRRL B-21554, and wherein said
6 toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

1 73. The method, according to claim 69, wherein said toxin is encoded by a nucleotide
2 sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer
3 pair selected from the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of
4 about 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ ID NOS.
5 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS. 21 and 25 to produce a
6 fragment of about 580 bp, and wherein said toxin does not have the amino acid sequence shown
7 in SEQ ID NO. 11.

1 74. The method, according to claim 69, wherein said toxin comprises a pesticidal
2 portion of the amino acid sequence shown in SEQ ID NO. 28, and wherein said toxin does not
3 have the amino acid sequence shown in SEQ ID NO. 11.

1 75. The method, according to claim 69, wherein said toxin comprises an amino acid
2 sequence which has at least about 75% homology with an amino acid sequence selected from
3 the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and
4 SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ
5 ID NO. 11.

1 76. The method, according to claim 69, wherein the full length of said toxin is
2 approximately 10-15 kDa.

1 77. The method, according to claim 69, wherein said toxin is encoded by a nucleotide
2 sequence which hybridizes under stringent conditions with a nucleotide sequence selected from
3 the group consisting of DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO.
4 5, and DNA which encodes SEQ ID NO. 7.

1 78. The method, according to claim 69, wherein said toxin immunoreacts with an
2 antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus*
3 *thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying
4 characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-
5 21553, and PS167H2 having the identifying characteristics of NRRL B-21554

1 79. The method, according to claim 69, wherein said toxin is encoded by a nucleotide
2 sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the
3 primer pair of SEQ ID NO. 29 and SEQ ID NO. 33.

1 80. The method, according to claim 69, wherein said toxin comprises a pesticidal
2 portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 32, SEQ
3 ID NO. 36, and SEQ ID NO. 41.

1 81. The method, according to claim 69, wherein said toxin comprises an amino acid
2 sequence which has at least about 75% homology with an amino acid sequence selected from
3 the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of
4 SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS
5 NO. 41.

1 82. The method, according to claim 69, wherein said pest is an insect.

1 83. The method, according to claim 82, wherein said insect is a coleopteran.

1 84. The method, according to claim 82, wherein said insect is a lepidopteran.

1 85. The method, according to claim 69, wherein said pest is a mite.

1 86. The method, according to claim 69, wherein said pest is corn rootworm.

1 87. The method, according to claim 69, wherein said toxin is encoded by DNA which
2 hybridizes with SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 37, or SEQ ID NO. 42, and
3 wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

1 88. The method, according to claim 69, wherein said toxin comprises the consensus
2 sequence shown in Figure 1 and wherein said toxin does not have the amino acid sequence
3 shown in SEQ ID NO. 11.

1 89. The method, according to claim 69, wherein said toxin comprises an amino acid
2 sequence which has at least about 75% homology with a pesticidal portion of an amino acid
3 sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO.
4 15, SEQ ID NO. 38, and SEQ ID NO. 43.

1 90. The method, according to claim 69, wherein said toxin comprises an amino acid
2 sequence which has at least about 90% homology with a pesticidal portion of an amino acid
3 sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO.
4 15, SEQ ID NO. 38, and SEQ ID NO. 43.

1 91. The method, according to claim 69, wherein said toxin comprises the consensus
2 sequence shown in Figure 1.

1 92. The method, according to claim 69, wherein said toxin comprises an amino acid
2 sequence which has at least about 75% homology with an amino acid sequence selected from
3 the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of
4 SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS
5 NO. 41.

1 93. The method, according to claim 69, wherein said toxin comprises an amino acid
2 sequence which has at least about 90% homology with an amino acid sequence selected from

3 the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of
4 SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS
5 NO. 41.

1 94. A method for controlling a non-mammalian pest wherein said method comprises
2 contacting said pest with a first toxin wherein said toxin has a characteristic selected from the
3 group consisting of:

- 4 (a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent
5 conditions with a nucleotide sequence selected from the group consisting of:
6 DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA
7 which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which
8 encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO.
9 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes
10 SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes
11 SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ
12 ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25,
13 SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of
14 SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ
15 ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin
16 does not have the amino acid sequence shown in SEQ ID NO. 11;
- 17 (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa
18 pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
19 selected from the group consisting of PS80JJ1 having the identifying
20 characteristics of NRRL B-18679, PS149B1 having the identifying
21 characteristics of NRRL B-21553, and PS167H2 having the identifying
22 characteristics of NRRL B-21554, and wherein said toxin does not have the
23 amino acid sequence shown in SEQ ID NO. 11;
- 24 (c) said toxin is encoded by a nucleotide sequence wherein a portion of said
25 nucleotide sequence can be amplified by PCR using a primer pair selected from
26 the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about
27 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ
28 ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS.
29 21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does
30 not have the amino acid sequence shown in SEQ ID NO. 11;

- 31 (d) said toxin comprises a pesticidal portion of the amino acid sequence shown in
32 SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence
33 shown in SEQ ID NO. 11;
- 34 (e) said toxin comprises an amino acid sequence which has at least about 75%
35 homology with a pesticidal portion of an amino acid sequence selected from the
36 group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID
37 NO. 38, and SEQ ID NO. 43; and wherein said toxin does not have the amino
38 acid sequence shown in SEQ ID NO. 11;
- 39 and further comprising contacting said pest with a second toxin having a characteristic selected
40 from the group consisting of:
- 41 (f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent
42 conditions with a nucleotide sequence selected from the group consisting of
43 DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and
44 DNA which encodes SEQ ID NO. 7;
- 45 (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa
46 pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
47 selected from the group consisting of PS80JJ1 having the identifying
48 characteristics of NRRL B-18679, PS149B1 having the identifying
49 characteristics of NRRL B-21553, and PS167H2 having the identifying
50 characteristics of NRRL B-21554;
- 51 (h) said toxin is encoded by a nucleotide sequence wherein a portion of said
52 nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID
53 NO. 29 and SEQ ID NO. 33;
- 54 (i) said toxin comprises a pesticidal portion of an amino acid sequence selected
55 from the group SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41; and
- 56 (j) said toxin comprises an amino acid sequence which has at least about 75%
57 homology with an amino acid sequence selected from the group consisting of
58 SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID
59 NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of
60 sequence IDS NO. 41.

1 95. The process, according to claim 94, wherein said pest is selected from the group
2 consisting of insects and mites.

1 96. The process, according to claim 95, wherein said pest is a coleopteran.

1 97. The process, according to claim 94, wherein said first toxin has a full length of
2 about 40-50 kDa and said second toxin has a full length of about 10-15 kDa.

1 98. A recombinant host transformed to express a toxin having activity against a non-
2 mammalian pest wherein said toxin has at least one characteristic selected from the group
3 consisting of:

- 4 (a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent
5 conditions with a nucleotide sequence selected from the group consisting of:
6 DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA
7 which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which
8 encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO.
9 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes
10 SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes
11 SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ
12 ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25,
13 SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of
14 SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ
15 ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin
16 does not have the amino acid sequence shown in SEQ ID NO. 11;
- 17 (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa
18 pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
19 selected from the group consisting of PS80JJ1 having the identifying
20 characteristics of NRRL B-18679, PS149B1 having the identifying
21 characteristics of NRRL B-21553, and PS167H2 having the identifying
22 characteristics of NRRL B-21554, and wherein said toxin does not have the
23 amino acid sequence shown in SEQ ID NO. 11;
- 24 (c) said toxin is encoded by a nucleotide sequence wherein a portion of said
25 nucleotide sequence can be amplified by PCR using a primer pair selected from
26 the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about
27 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ
28 ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS.

- 29 21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does
30 not have the amino acid sequence shown in SEQ ID NO. 11;
- 31 (d) said toxin comprises a pesticidal portion of the amino acid sequence shown in
32 SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence
33 shown in SEQ ID NO. 11;
- 34 (e) said toxin comprises an amino acid sequence which has at least about 75%
35 homology with a pesticidal portion of an amino acid sequence selected from the
36 group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID
37 NO. 38, and SEQ ID NO. 43; and wherein said toxin does not have the amino
38 acid sequence shown in SEQ ID NO. 11;
- 39 (f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent
40 conditions with a nucleotide sequence selected from the group consisting of
41 DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and
42 DNA which encodes SEQ ID NO. 7;
- 43 (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa
44 pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
45 selected from the group consisting of PS80JJ1 having the identifying
46 characteristics of NRRL B-18679, PS149B1 having the identifying
47 characteristics of NRRL B-21553, and PS167H2 having the identifying
48 characteristics of NRRL B-21554;
- 49 (h) said toxin is encoded by a nucleotide sequence wherein a portion of said
50 nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID
51 NO. 29 and SEQ ID NO. 33;
- 52 (i) said toxin comprises a pesticidal portion of an amino acid sequence selected
53 from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO.
54 41; and
- 55 (j) said toxin comprises an amino acid sequence which has at least about 75%
56 homology with an amino acid sequence selected from the group consisting of
57 SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID
58 NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of
59 sequence IDS NO. 41.

1 99. The recombinant host, according to claim 98, wherein said host expresses a first
2 toxin which has a characteristic selected from the group consisting of:

- 3 (a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent
4 conditions with a nucleotide sequence selected from the group consisting of:
5 DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA
6 which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which
7 encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO.
8 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes
9 SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes
10 SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ
11 ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25,
12 SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of
13 SEQ ID NO. 28, SEQ ID NO. 39, DNA which encodes SEQ ID NO. 38, SEQ
14 ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin
15 does not have the amino acid sequence shown in SEQ ID NO. 11;
- 16 (b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa
17 pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
18 selected from the group consisting of PS80JJ1 having the identifying
19 characteristics of NRRL B-18679, PS149B1 having the identifying
20 characteristics of NRRL B-21553, and PS167H2 having the identifying
21 characteristics of NRRL B-21554, and wherein said toxin does not have the
22 amino acid sequence shown in SEQ ID NO. 11;
- 23 (c) said toxin is encoded by a nucleotide sequence wherein a portion of said
24 nucleotide sequence can be amplified by PCR using a primer pair selected from
25 the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about
26 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ
27 ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS.
28 21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does
29 not have the amino acid sequence shown in SEQ ID NO. 11;
- 30 (d) said toxin comprises a pesticidal portion of the amino acid sequence shown in
31 SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence
32 shown in SEQ ID NO. 11; and
- 33 (e) said toxin comprises an amino acid sequence which has at least about 75%
34 homology with an amino acid sequence selected from the group consisting of
35 SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ

36 ID NO. 43; and wherein said toxin does not have the amino acid sequence
37 shown in SEQ ID NO. 11;

38 and said host expresses a second toxin having a characteristic selected from the group consisting
39 of:

- 40 (f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent
41 conditions with a nucleotide sequence selected from the group consisting of
42 DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and
43 DNA which encodes SEQ ID NO. 7;
- 44 (g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa
45 pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate
46 selected from the group consisting of PS80JJ1 having the identifying
47 characteristics of NRRL B-18679, PS149B1 having the identifying
48 characteristics of NRRL B-21553, and PS167H2 having the identifying
49 characteristics of NRRL B-21554;
- 50 (h) said toxin is encoded by a nucleotide sequence wherein a portion of said
51 nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID
52 NO. 29 and SEQ ID NO. 33;
- 53 (i) said toxin comprises a pesticidal portion of an amino acid sequence selected
54 from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO.
55 41; and
- 56 (j) said toxin comprises an amino acid sequence which has at least about 75%
57 homology with an amino acid sequence selected from the group consisting of
58 SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID
59 NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of
60 sequence IDS NO. 41.

1 100. The recombinant host, according to claim 98, wherein said host is selected from
2 the group consisting of plants, yeasts, and bacteria.

1 101. The isolated polynucleotide, according to claim 1, wherein said nucleotide
2 sequence hybridizes with SEQ ID NO. 37.

1 102. The isolated polynucleotide, according to claim 1, wherein said nucleotide
2 sequence hybridizes with SEQ ID NO. 42.

1 103. The purified toxin, according to claim 31, which is encoded by DNA which
2 hybridizes with SEQ ID NO. 37.

1 104. The purified toxin, according to claim 31, which is encoded by DNA which
2 hybridizes with SEQ ID NO. 42.

1 105. The isolated polynucleotide, according to claim 24, wherein said toxin comprises
2 the amino acid sequence shown in SEQ ID NO. 36.

1 106. The isolated polynucleotide, according to claim 24, wherein said toxin comprises
2 the amino acid sequence shown in SEQ ID NO. 41.

1 107. The purified toxin, according to claim 56, wherein said toxin comprises the amino
2 acid sequence shown in SEQ ID NO. 36.

1 108. The purified toxin, according to claim 56, wherein said toxin comprises the amino
2 acid sequence shown in SEQ ID NO. 41.

1 109. An isolated polynucleotide comprising a nucleotide sequence which encodes an
2 approximately 10-15 kDa 80JJ1 toxin active against non-mammalian pests, wherein said
3 nucleotide sequence has been optimized for expression in plants.

1 110. The isolated polynucleotide, according to claim 109, wherein said polynucleotide
2 comprises the sequence shown in SEQ ID NO. 44.

1 111. An isolated polynucleotide comprising a nucleotide sequence which encodes an
2 approximately 40-50 kDa 80JJ1 toxin active against non-mammalian pests, wherein said
3 nucleotide sequence has been optimized for expression in plants.

1 112. The isolated polynucleotide, according to claim 111, wherein said polynucleotide
2 comprises the sequence shown in SEQ ID NO. 45.

1/2

FIG. 1

	1				50
{149b145k}GLYAA	TYLSLDDSGV	SLMNKNDDDI	DDYNLKWFLF	
{167h245k}HAA	TYLSLDDSGV	SLMNKNDDDI	DDYNLRWELF	
{80jj145k}	MLDTNKVYEI	SNLANGLYTS	TYLSLDDSGV	SLMSKKDEDI	DDYNLKWELF
Consensus	-----	-----	-----	-----	-----
	51				100
{149b145k}	PIDDDQYIIT	SYAANNCKVW	NVNNDKINVS	TYSSTNSIQK	WQIKANGSSY
{167h245k}	PIDDNQYIIT	SYAANNCKVW	NVNNDKINVS	TYSSTNSIQK	WQIKANASSY
{80jj145k}	PIDNNQYIIT	SYGANNCKVW	NVKNDKINVS	TYSSTNSVQK	WQIKAKDSSY
Consensus	PID--QYIIT	SY-ANNCKVW	NV-NDKINVS	TYSSTNS-QK	WQIKA--SSY
	101				150
{149b145k}	VIQSDNGKVL	TAGTGQALGL	IRLTDESSNN	PNQQWNLTSP	QTIQLPQKPI
{167h245k}	VIQSNGKVL	TAGTGQSLGL	IRLTDESPDN	PNQQWNLTSP	QTIQLPQKPT
{80jj145k}	IIQSDNGKVL	TAGVGQSLGI	VRLTDEEPEN	SNQQWNLTSP	QTIQLPQKPK
Consensus	-IQS-NGKVL	TAG-GQ-LG-	-RLTDE---N	-NQQWNLT-V	QTIQLP-KP-
	151				200
{149b145k}	IDTKLKDYPK	YSPTGNIDNG	TSPQLMGWTL	VPCIMVNDPN	IDKNTQIKTT
{167h245k}	IDTKLKDYPK	YSQTGNIDKG	TPPQLMGWTL	IPCIMVNDPN	IDKNTQIKTT
{80jj145k}	IDDKLKDHPE	YSETGNINPK	TTPQLMGWTL	VPCIMVNDSP	IDKNTQIKTT
Consensus	ID-KLKD-P-	YS-TGNI---	T-PQLMGWTL	-PCIMVND--	IDKNTQIKTT
	201				250
{149b145k}	PYYILKKYQY	WQRAVGSNVA	LRPHEKKSYS	YEWGTEIDQK	TTIINTLGFG
{167h245k}	PYYILKKYQY	WQQAAGSNVA	LRPHEKKSYS	YEWGTEIDQK	TTIINTLGFG
{80jj145k}	PYYIFKKYKY	WNLAKGSNVS	LLPHQKRSYD	YEWGTEKNQK	TTIINTVGLQ
Consensus	PYYI-KKY-Y	W--A-GSNV-	L-PH-K-SY-	YEWGTE--QK	TTIINT-G-Q
	251				300
{149b145k}	INIDSGMKFD	IPEVGGGTDE	IKTQLNEELK	IEYSHETKIM	EKY.....
{167h245k}	INIDSGMKFD	IPEVGGGTDE	IKTQLNEELK	IEYSRETAKIM	EKY.....
{80jj145k}	INIDSGMKFE	VPEVGGGTED	IKTQLTEELK	VEYSTETKIM	TKYQEHSEID
Consensus	INIDSGMKF-	-PEVGGGT--	IKTQL-EEELK	-EYS-ETKIM	-KY-----
	301				350
{149b145k}
{167h245k}
{80jj145k}	NPTNQPMNSI	GLLIYTSLEL	YRYNGTEIKI	MDIETSDHDT	YTLTSPYNNH
Consensus	-----	-----	-----	-----	-----
	351				386
{149b145k}
{167h245k}
{80jj145k}	EALLLLTNHS	YEEVEEITKI	PKHTLIKLLK	HYFKK.
Consensus	-----	-----	-----	-----	-----

2/2

FIG. 2



